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THE ETIOLOGY OF GRANULOPENIA (AGRANULOCYTOSIS)*

WITH PARTICULAR REFERENCE TO THE DRUGS CONTAINING THE BENZENE RING

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THE disease known as agranulocytosis was described as a clinical entity only eleven years ago.[1] The first case in the United States was reported nine years ago.[2] Since that time it has been constantly on the increase and at this time we have collected 473 cases from the American and Canadian literature alone. It is apparently increasing in frequency and also being recognized more readily.

In our review of the American cases we have been impressed by the consistently increasing number of the acute fulminant types and also by the large number of so-called chronic granulopenic patients as reported by Roberts and Kracke.[3]

It is quite probable that the so-called acute granulopenia in which the granulocytes entirely disappear from the peripheral blood represents only a fraction of the total number of individuals whose blood count is depressed to some degree. Although there was a sporadic case of this disease prior to 1922, it certainly must have occurred very infrequently.

Pepper[4] believes that it was quite common many years ago and cites the numerous instances of so-called putrid sore throat or malignant angina. But

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this assumption is entirely unwarranted since the single diagnostic criterion, namely, a low leucocyte count, was lacking in these cases. The disease is one that is easily diagnosed, runs a dramatic and clear-cut clinical course, and the fact that it was not observed before 1922 is practical assurance that it did not exist before that time. To state otherwise would be to reflect on the diagnostic acumen of our clinicians of the past. Blood counts have been taken for fifty years. We must consider, then, that granulopenia is a disease of modern times and one that has come among us during the past ten years.

ETIOLOGY

Live Bacteria.—Since the first report of Schultz[1] in Germany and the report of Lovett[2] in the United States, many investigators have directed their efforts toward the solution of the etiologic factors. Up to this time these efforts have met with very little or no success. It is the chief purpose of this paper to present briefly a summation of the work in this field and to formulate an hypothesis for the cause of granulopenia and to present evidence supporting this view.

Lovett[2] noted in her case the presence of mouth ulcers infected with *B. pyocyaneus* and at once suspected this organism as etiologic. She injected numerous laboratory animals but failed to produce the condition.

Similar observations have been made by Linthicium,[5] Windham,[6] Keeney.[7] Also, Friedmann[8] in a study of 23 cases found *B. pyocyaneus* in the blood stream of one. It will be noted that this same organism has been found in the blood stream of three of the American cases (Table I). These findings have led many to suspect it as etiologic but up to this time efforts to reproduce the disease in laboratory animals with it, have been unsuccessful. There is little support for the bacterial etiology of granulopenia since it has been well demonstrated by Roberts and Kracke[9] that the basic pathology is first, the disappearance of the neutrophiles, and this in turn is followed by the invasion by any and every organism that is accessible, particularly throughout the entire length of the gastrointestinal tract. Most writers are in accord with the conception that granulopenia is first a disease of the bone marrow, followed by disappearance of peripheral granulocytes, this in turn followed by varying degrees and types of infectious processes.

It will be noted from a study of Table I that at least 25 organisms have been found as blood stream invaders in this disease and that approximately 20 per cent of the cases show blood stream infection. The wide variety of invading bacteria can also be noted from the numerous types that are responsible for the ulcers. A few reports tending to incriminate various organisms have been made. Notable among these is the work of Fried and Dameshek[9a] in which they reported the production of primary granulopenia in rabbits by the intravenous injection of *Salmonella Suipestifer*. An analysis of their report shows that in no instance did they reproduce the condition as it is observed clinically, since they failed to produce a sustained and prolonged depression of the leucocyte count, but did produce a temporary neutropenia which, as has been well demonstrated, will follow the injection of any killed organisms or other matter in a finely divided state.

TABLE I

BLOOD STREAM INFECTION IN GRANULOPENIA

ORGANISM	NUMBER OF CASES
Streptococcus hemolyticus	8
Streptococcus viridans	15
Streptococcus (type undetermined)	9
Staphylococcus albus	3
Staphylococcus aureus	4
Bacillus Friedlander	4
Pneumococcus, Type 2	3
Pneumococcus, Type 3	1
Pneumococcus, Type 4	2
Pneumococcus, Type undetermined	2
Diplococcus (undetermined)	4
Bacillus pyocyaneus	3
Bacillus paratyphosus B.	1
Bacillus coli	3
Bacillus proteus	1
Streptothrix	3
Streptococcus with other bacteria	9
Fusiform bacillus (Gram negative)	1
Estivo-autumnal parasites	1
Varines cocci	3
Staphylococcus (undetermined)	1
Diphtheria bacillus	1
Vincent's organisms	1
Typhoid bacillus	1
Bacillus (undetermined)	1
Total number of positive cultures	74
Negative, or not stated	395

1. Blood cultures are positive in about 20 per cent of the total number of cases or positive in 70 per cent of the examined cases.
2. The streptococci are the most frequent invaders.
3. The blood stream infection is probably secondary to the leucopenic state.

The clinical onset of many cases of granulopenia has been preceded by tooth extractions, treatment for pyorrhea, mouth ulcers, etc. It will be noted in a study of Table II that this is a common history and in many of these cases Vincent's organisms have been isolated. We believe that these oral

TABLE II

IMPORTANT FEATURES OF THE HISTORIES

Tooth extraction	27
Treatment for pyorrhea, etc.	22
Treatment for syphilis	22
Influenza (mild)	17
Tonsillectomy	5
Sore throat	85
Acute coryza	15
Malaise and weakness	91
Sudden onset (no premonitory symptoms)	48
Various operations	8
Abscesses and furunculosis	7
Arthritis	6
93 other clinical conditions, 1 to 2 in each patient	
Not stated adequately	77

1. Whether or not the numerous instances of dental therapy preceding the disease are the results of a previously unrecognized leucopenia has not been settled.
2. Nearly three-fourths of all reported cases had been receiving treatment for some other disease for variable periods prior to their attacks of granulopenia.
3. It is interesting that eight patients developed the disease while in a hospital for treatment for other conditions.

manifestations are secondary to a preceding neutropenic state. It must be borne in mind that when the granulopenic patient consults a physician he presents the terminal stage of a condition that has existed possibly for many months. And, furthermore, many people have developed acute fulminant granulopenia and have died without showing any ulcers whatever. There has been no experimental or clinical evidence to indicate that Vincent's organisms are capable of producing a severe and sustained leucopenic state.

There are many reports in the literature concerning the efforts of various investigators to reproduce the disease in laboratory animals with organisms that have been isolated from their respective patients, but all of this work has been unsuccessful. Piersol and Steinfeld[10] injected intravenously into rabbits inactivated cultures, Berkefeld filtrates, and supernatant fluids of cultures of many organisms and failed to reproduce the condition and called attention to the fact that a temporary leucopenia may be produced by the injection of peptones and a large number of other proteins.

It has been stated that granulopenia may be due to the continued absorption of toxins from sites of focal infection. The recent work of Dennis[11] is important. He was able to produce a sustained and marked leucopenia in rabbits in which he allowed the *Streptococcus viridans* to grow in the tissue under such conditions that the toxins were diffused throughout the animal body while the organisms remained in situ. His results were not conclusive in the use of *Staphylococcus aureus, Streptococcus hemolyticus,* and *B. proteus.* It will be noted from a study of Table I that *Streptococcus viridans* has been reported as the most frequent blood stream invader in this disease. Dennis[12] stated later that a weakness of his experimental work consisted in the use of a very large dose in relation to the size of the animal. It has long been known, of course, that overwhelming infection with certain organisms will produce variable degrees of leucopenia.

Dead Bacteria.—The injection with dead bacteria in the course of vaccination has been suggested as a possible cause. Bromberg and Murphy[13] reported a typical case following a series of typhoid vaccine injections. Kracke[14] has reported a similar case in which, however, the patient had taken so much phenacetine during the previous four-year period that she was suffering from a severe sulphemoglobinemia. Dr. R. P. Irwin[15] of Moulton, Ala., has submitted a record of a typical case following typhoid vaccination. In his case, also, the patient was under treatment for various other conditions in which she was given considerable medication of various types. Pepper[4] has advanced the conception that the disease may be allergic in nature, but there is little evidence to support his view.

Glandular Dysfunction.—The influence of the endocrine glands on the output of both granulocytes and erythrocytes has been studied considerably and has long been suspected.

Hubble[16] summarizes this work and postulates that bone marrow depression may be caused by a pituitary basophilic insufficiency, and he states that there is much evidence to indicate that granulopenia may be caused by cortical adrenal dysfunction.

Britton and Corey[17] have reported that experimental adrenal insufficiency in cats resulted in a marked degree of neutropenia with the return of the granulocytes to normal following administration of corticoadrenal extract. They did not produce, however, the typical granulopenic syndrome as is commonly seen.

Jaffe[18] has studied a patient with generalized infiltration of lymphadenoma which included invasion and destruction of the adrenal cortex with the patient finally dying with a typical granulopenia. He infers from this that the granulopenia was due to a cortical adrenal insufficiency.

Kunde et al.[19] have noted the production of a hypoplastic and aplastic bone marrow in thyroidectomized rabbits. Evidence accumulated indicates that the ductless glands in their various relationships have an effect on the output of blood cellular elements. This conception, however, will have to await further confirmation.

Radiation.—Excessive exposure to radiation has been suspected as a possible etiologic factor. It is known that Thorium X will produce a marked neutropenia in animals and in the human being. It is conceivable, of course, that excessive roentgen radiation can produce marked bone marrow depressions, and it should be used with caution in the therapy of this disease. Also, it will be noted from a study of Table III that granulopenia is a disease essentially of the white race, being quite rare in negroes, and this may have some relationship to the radiation theory.

Bone Marrow Idiosyncrasy.—This rather ill-defined conception includes the opinions of a considerable number of writers all of whom postulate that an occasional individual has a weakened or faulty bone marrow which will become nonfunctional if submitted to undue strain or stimulation.

Beck,[20] in her excellent review of this disease, holds the view that the primary pathology will not be found in the marrow itself, but in that particular organ or tissue which regulates granulopoiesis. She believes that those cases showing peripheral neutropenia but with a normal bone marrow would

TABLE III

THE INCIDENCE OF GRANULOPENIA

Number of cases in the United States	473
Number of cases in the white race	442
Number of cases in the colored race	8
Race not stated	23
Number of cases in males	161
Number of cases in females	310
Sex not stated	2

1. The disease is essentially one of the white race.
2. It exists in the ratio of two females to one male.

be due to a lack of the chemotactic factor, whereas the damaged bone marrow would be due to a lack of the maturation factor. As Beck infers, it is true that the bone marrow in some cases, both at autopsy and sternal biopsy, shows a normal or hyperplastic state while other cases show a definite aplasia of the marrow.

Fitzhugh and Krumbhaar[21] are of the opinion that the bone marrow may be hyperplastic up to the myelocytic level but that maturation ceases at that point.

Kracke[22] has studied a case which showed a definite hyperplastic bone marrow at sternal puncture at a time when the peripheral cell count was 500. This was explained four days later when the count reached 25,000. Finally this patient died with a second attack, showing a markedly aplastic bone marrow at autopsy.

If Beck's conception is correct, it follows that there is sometimes a temporary restriction of either the maturation or the chemotactic factor. Her theory, however, does not explain why such derangement occurs.

Chemicals.—It has long been known that various chemicals will depress the marrow function, resulting in complete inhibition of all cellular types. For example, benzene poisoning has been recorded as a process in which hematopoiesis is completely inhibited in all of the three bone marrow elements, resulting in a picture simulating aplastic anemia. It has been shown by Kracke[23] that benzene may be introduced into the rabbit in such small doses that it exerts a selective affinity for the granulopoietic tissue only, leaving the erythrocytic and thrombocytic elements relatively undisturbed. This same situation is conceivably true with other bone marrow depressing agents, and, no doubt, is true in the case of arsphenamine. Arsphenamine poisoning is a known etiologic agent in the production of bone marrow depression. The clinical picture resulting has been chiefly one of anemia, associated with granulopenia and thrombocytopenia as well. It will be noted from a study of Table II that at least 22 of the cases of granulopenia have been preceded by arsphenamine therapy, and there is little doubt that the drug was etiologic in that particular group of cases. But still more important, it shows that arsphenamine is capable of producing a type of bone marrow depression restricted chiefly to granulopoiesis to such an extent that the clinical syndrome resulting cannot be differentiated from true idiopathic granulopenia.

It has been shown that the injection of the human being with certain preparations of gold will produce a condition closely simulating, if not identical, with true granulopenia. Many such cases have been reported from France, Achard et al.,[24] Ameuille and Braillon,[25] Angeras and Ginsbourg.[26]

The fact that typical granulopenia can be produced with gold preparations and with arsphenamine preparations which contain the benzene ring is evidence that this class of products can, and in some instances does, manifest a selective action on the granulopoietic tissue only. It seems, therefore, in the study of etiology that it becomes our problem to determine which of these chemicals has come into wider usage and closer contact with our people during the last ten-year period or during the period of agranulocytosis.

THE INCIDENCE OF GRANULOPENIA

Our review of the reported cases in the United States and Canada has uncovered many interesting findings in connection with the occurrence of this disease.

Geographic Distribution.—We have collected from the literature reports of 473 cases from 1922 to 1932 inclusive. There has been an increasing number reported each year, probably because of the larger number of cases, and its more widespread recognition. During this same period there have been reported in Germany approximately 350 cases. The French literature reveals about 100 cases in that country. The disease is apparently quite rare in Russia, Poland, and the Far East, and, for some unknown reason, it is extremely rare in Great Britain. It may be more prevalent in the Far East than we suspect, but the cases probably are not reported.

Dennis[12] has observed two cases in the University Hospital at Beirut, Syria, and states that he discussed the incidence of the disease with several physicians from Palestine and Egypt, and practically all of them had seen cases. Dr. Kleeberg who is practicing in Jerusalem said he had observed the condition there. Up to 1929 there had been only three cases reported from Russia. The rarity of the disease in England is of special significance. It is quite evident that it does not occur in that country to any appreciable extent. As we shall attempt to show, this may have its cause in the type of drugs that are employed by physicians of Great Britain. Briefly stated, granulopenia occurs mainly in the United States and Germany (see Table IV).

TABLE IV

GEOGRAPHIC DISTRIBUTION OF GRANULOPENIA (FROM 1922 TO 1932)

	NUMBER OF CASES
United States	473
Germany	350 (Approx.)
France	100 (Approx.)
England	6 (Total)
Italy	50 (Approx.)

1. The disease is extremely prevalent in Germany and the United States.
2. It is extremely rare in England.

Age, Sex, and Race.—A study of Table III reveals the highly significant finding that granulopenia is a disease primarily of the white race, since only eight reports of its occurrence in the colored race were found, and in the

TABLE V

THE SEASONAL INCIDENCE

MONTH	NUMBER OF CASES
January	32
February	27
March	39
April	29
May	26
June	32
July	23
August	26
September	17
October	25
November	28
December	26
Not stated	159

1. There is no apparent seasonal variation.

majority of these, the onset was preceded by arsphenamine therapy. It occurs in the ratio of two females to one male, and this is also true of the cases from other countries. It has no particular seasonal incidence as shown by Table V as cases occur in about equal number throughout the months of the entire year.

The average age is between forty and fifty and ranges from under one year of age to above eighty. It is, therefore, a disease chiefly of middle life.

Occupation.—In our studies we have paid particular attention to the occupation of the reported cases and this study has revealed some very interesting and valuable information. It is a striking fact that granulopenia is more prevalent among physicians and their relatives, nurses, hospital employees, and medical students than in any other group of people. Based on the 1930 population census of the United States, it occurs fifty times more frequently in physicians than in lawyers, and two hundred times more frequently in nurses than in female school-teachers. We have made this observation before and have called attention to this singular finding.

It will be noted that of the 200 reported cases in which the occupation is stated, including the housewives, approximately 10 per cent of these have been physicians and members of the medical group. The so-called medical group, including physicians, nurses, hospital employees, etc., constitute 60 per cent of the reported cases of agranulocytosis in the United States in that group in which the occupation is definitely stated. It is not justified to explain this on the belief that this illness is diagnosed more promptly or efficiently in this group of people, since it is well known that physicians are most negligent concerning their own health.

This observation has been noted by several writers. In 1931 Stellhorn and Amolsch[27] in summarizing 42 cases of granulopenia from the region about Detroit, Michigan, made this significant statement, "It is rather curious to note that many of these patients were members of the medical or allied professions, or were relatives of physicians." In their series of cases were 2 nurses, 2 physicians, 1 dentist, and 7 immediate relatives of physicians. Studies of the reports of large series of cases show this same finding to be invariably true. In Harkin's[28] group of 8 cases there was one doctor, one medical student, and one nurse. In a series of about 15 patients from the Mayo Clinic[29] the 2 cases of typical acute fulminant granulopenia occurred in a doctor and a nurse. Hinton[30] and his associates in Macon, Georgia, have studied 6 cases in that section, of which one was a physician and one was a nurse. Sydenstricker[31] reported one case from Augusta, Georgia, who was a physician. Madison and Squier[32] have recently reported from Milwaukee, Wisconsin, a valuable observation, in which they studied 14 patients, of whom 5 were physicians, physicians' wives and nurses, and they stated, "In this group as in the disease at large, there seems to be a most remarkable relation to the medical and allied professions." Fitzhugh and Comroe[33] reported 18 cases from the University of Pennsylvania, and one of them is listed as a physician. Kracke[34] has studied 11 cases around Atlanta, Georgia, in which

there was one physician, one nurse, and one dentist's wife. It is interesting that the only case report we have noted from Poland was one of a physician. Furthermore, we have been advised[12] that 2 physicians are included in the small group of cases around Denver, Colorado.

These observations leave little doubt but that granulopenia is a disease which is peculiar in its relation to the medical profession. Our chief problem then is to determine the possible etiologic factors that could enter into the lives of people in the medical and allied professions that would produce such a large number of cases in this group (see Table VI).

TABLE VI

STUDIES ON OCCUPATION

Housewives	102
Physicians	22
Nurses	17
Maids in hospital	4
Medical students	3
Laboratory technician	1
Relatives of physicians	11
School-teachers	2
Farmers	5
Business men	10
Clerks	2
Sailors	3
Dentist	1
49 other occupations	1 each
Occupation not stated	289

1. The disease is extremely prevalent in physicians and their relatives, nurses, and hospital employees.
2. It is fifty times more prevalent in physicians than in lawyers.
3. It is two hundred times more prevalent in nurses than in female school-teachers. (Based on population from U. S. Census.)

Class of People.—Kracke[14, 23, 34, 35] has repeatedly called attention to the fact that this disease occurs in the better class of our people. In 11 cases in the Atlanta section were included the following: (1) A retired, elderly, cultured woman; (2) a retired, elderly, wealthy woman; (3) a wife of a leading business man in a small town in Georgia; (4) a chief of police in a Georgia city; (5) a wife of an Atlanta dentist; (6) chief librarian in a girls' college; (7) a cultured, refined widow who was economically, moderately well-to-do; (8) the wife of a leading Atlanta architect; (9) a practicing physician; (10) a nurse in the University hospital; (11) a middle-aged white man in the municipal hospital of Atlanta (who was a patient for two months there before developing the disease).

In this connection we quote from the report of Stellhorn and Amolsch[27] as follows: "Very few of the cases occurred among the poor classes. Receiving Hospital, a large hospital maintained by the City of Detroit for the poor, and having an excellent staff of alert, well-read physicians, has no record of any cases of primary granulocytopenia. At least 9 of 31 true cases are extremely well-to-do. Locally the disease is most common among the people of the better classes."

To summarize the peculiar distribution of granulopenia based on our present observations and an exhaustive review of the literature, we would

state: that it is a disease which definitely occurs in the better class of our people; that it has a peculiar predilection for members of the medical and allied professions; that it is confined almost exclusively to the white race; and that it occurs chiefly in Germany and the United States.

THE RELATION OF DRUG THERAPY TO GRANULOPENIA

We have long felt that the peculiar distribution of granulopenia could well be correlated with the use of certain benzene containing drugs that are commonly used for therapeutic purposes. We called attention to this in June, 1930, at which time we believed that the benzene ring was incriminated in the production of this disease. At that time we reported our efforts to produce experimentally granulopenia in rabbits using some of the various oxidation products of benzene and using, also, a series of drugs which contained the benzene ring as their nucleus. We have investigated as carefully as possible the various etiologic factors in this respect in eleven cases of true idiopathic granulopenia with the following findings:

CASE 1.—An elderly white woman who had been subjected to repeated cystoscopic examinations over a period of nearly a year, during which time she was given frequently a large number of Peralga tablets (which contain 71 per cent of amidopyrine).

CASE 2.—A middle-aged white woman in whom the onset was preceded by a protracted period of illness diagnosed as influenza, during which time she was given practically daily amidopyrine and empirin compound for a period of several weeks; and prior to that time she had taken about three tablets two or three times a month over the past four-year period.

CASE 3.—A middle-aged white man had taken for more than a year, as much as 15 gr. of amidopyrine daily for attacks of precordial pain, interspersed with a prescription containing phenacetine. Just before the clinical onset he was treated for a condition diagnosed as a mild attack of influenza and was given a prescription of capsules containing phenacetine.

CASE 4.—A middle-aged white woman, the wife of a dentist, had taken a proprietary preparation known as James' Tablets several times weekly over a period of from five to ten years, according to her husband. He stated that his wife took these tablets for every ache and pain. Investigation showed that each tablet contained one grain of acetanilid.

CASE 5.—A young white woman, a nurse in the Emory University Hospital, had taken from 5 to 15 gr. of amidopyrine daily during her menstrual periods for a period of several years and had taken them more frequently during the seven or eight weeks preceding the onset of her illness.

CASE 6.—A middle-aged white woman, librarian, had emotional stress during her last year of life. She was known to have suffered from profuse menstruation and, according to such information as was available, was thought to have been addicted to the use of the so-called coal tar drugs.

CASE 7.—A middle-aged white woman had received three doses of neoarsphenamine and had taken empirin compound for her various minor symptoms (phenacetine, aspirin and caffeine).

CASE 8.—An elderly white woman gave a history of prolonged use of compounds containing phenacetine which had been prescribed for her by her dentist in connection with a long period of dental work.

CASE 9.—A middle-aged white woman had been given phenacetine for five years preceding the onset of her illness, and during this five-year period had taken so much that when she was admitted to the hospital with granulopenia, she presented a typical picture of phenacetine poisoning with the marked slate blue color of sulphemoglobinemia.

CASE 10.—A middle-aged physician in general practice was known by his brother, who was a dentist, to be a coal tar drug addict, to such an extent, that his brother had even at

times remonstrated that he took entirely too many of these drugs. His brother stated that he had taken such drugs as empirin compound, allonal, pyramidon, etc., almost daily for years for the slightest ache or pain.

CASE 11.—A middle-aged white man admitted to Grady Hospital (Municipal) Atlanta, for treatment of rectal fistula. He was in the hospital for two months during which time his medication covered a wide range of drugs. Included in this were generous doses of pyramidon. It is not known to what extent he took these preparations before his admission to the hospital. It is noteworthy that this patient developed granulopenia only after he had been in the hospital for two months and is the only case of granulopenia that has been observed in the 600 bed Municipal Hospital of Atlanta.

The cases outlined above were of the true idiopathic granulopenic type and do not include the chronic forms.

We have felt for the past three years that members of the medical profession have been prone to prescribe and use this class of drugs for their own ailments and for members of their families. We also feel that the average hospital nurse in the course of her duties, when seeking a mild analgesic, will, because of her familiarity with these newer preparations, employ one of the coal tar drugs instead of the older remedies such as aspirin. We feel also, that average laymen when treating their own aches and pains will use aspirin unless they utilize some drug that has been prescribed for them by their physicians. We have further observed that the average negro who, incidentally, is not subject to as many minor complaints and illnesses as the white person, knows little about this class of drugs and seldom, if ever, uses them on his own initiative.

In order to verify these points, we have conducted a study of several of these groups with particular reference to the usage of the coal tar drugs. This information was obtained by history blanks which were filled out by the following groups of people: (1) 154 student and graduate nurses; (2) 100 physicians; (3) 100 random admissions to the University Hospital (white); (4) 100 admissions to the Municipal Hospital (colored). The results obtained from this series of questionnaires will be seen in Table VII.

To summarize the information from this table, it will be noted that of those nurses who resort to the use of drugs, 76 per cent will select a drug containing the benzamine group; second, of those physicians who will prescribe for themselves and their own families, 56 per cent will use drugs of the benzamine group; third, of the average white lay patients, approximately 27 per cent will select drugs of this class; and, in the colored group about 15 per cent would use this type of drug.

TABLE VII

	NURSES	DOCTORS	LAITY	NEGROES
Total number of histories	154.0	100	100.0	100
Total number using drugs	124.0	100	65.0	22
Number using benzamine drugs	94.0	55	17.0	15
Percentage using benzamine drugs	75.8	55	26.1	15
Number using acetylsalicylic acid	26.0	45	44.0	7
Percentage using acetylsalicylic acid	20.8	45	67.9	7
Number using drugs not classified above	4.0	--	4.0	--
Per cent using drugs not classified above	3.3	--	6.0	--

It can be seen, therefore, that there exists almost a direct proportion between the incidence of granulopenia in these groups and the incidence of drug

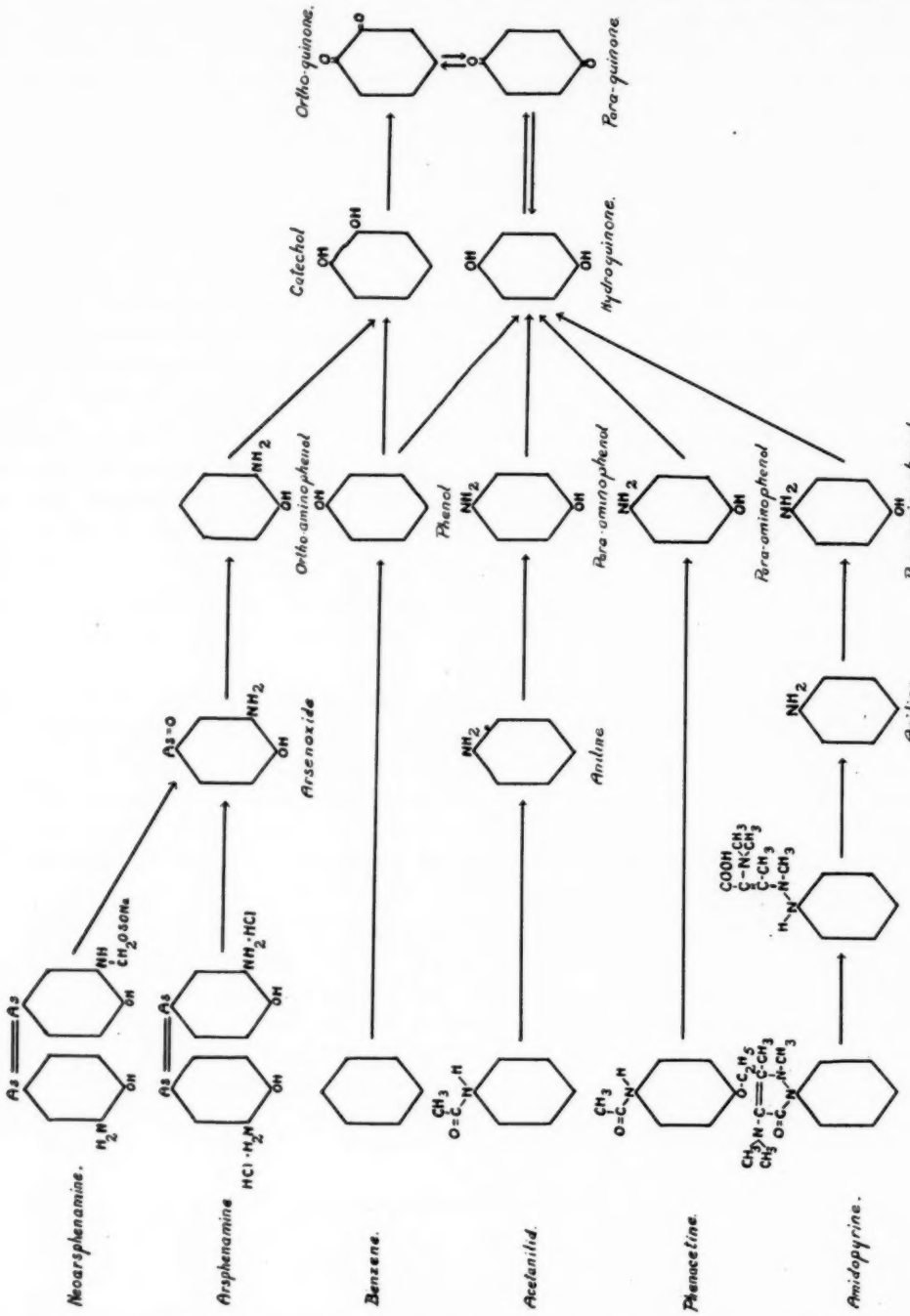


Fig. 1.—Possible oxidation reactions of the benzamine group of drugs and of benzene.

usage in the same groups. In other words, if a nurse needs an analgesic 3 out of 4 will select a coal tar drug and 3 physicians out of 5 will use the same class of drug, whereas, only 1 white layman out of 4 and 1 negro out of 8

will use the benzamine drugs. We believe, therefore, that there exists a direct relationship between the usage of these drugs to the incidence of granulopenia in these various groups.

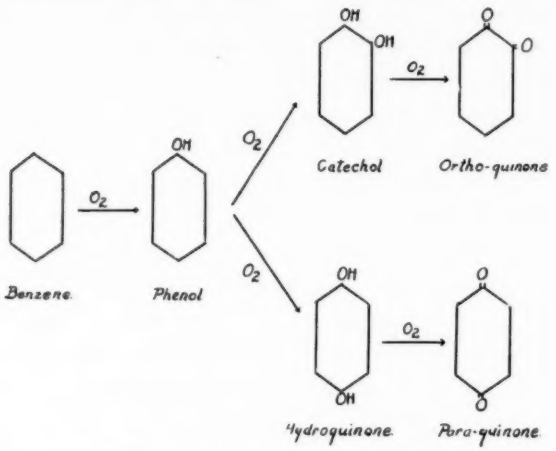
The peculiar geographic distribution of granulopenia can also be correlated with usage of this class of drugs. The prevalence of the disease in Germany and the United States approximates the use of coal tar derivatives of this class in these two countries. The practical absence of the disease in England is correlated with the fact that this class of drugs is used to a lesser extent in that country. It is also known that some of these drugs, notably those in combination with barbiturates, have been introduced only within the last ten- or twelve-year period which is the time period of granulopenia. Therefore, based upon these statistical studies and upon our observations in a series of 11 cases of granulopenia and on our studies of drug usage in these various groups of people, we believe that this must be seriously considered as an etiologic factor in this disease.

In October, 1933, Madison and Squier[32] presented a report of 14 cases of granulopenia, and in every instance the onset of the illness had been preceded by prolonged or intensive administration of drugs of this class, and they state, "In each of the 14 cases there was a definite history of amidopyrine in combination with a barbiturate or pyramidon in practically all of the cases we have seen."

On the basis of the evidence submitted in this paper we, therefore, have formulated the hypothesis that the disease owes its high incidence in nurses and physicians to their indiscriminate use of drugs containing the benzamine group, the more prominent members of which are shown in Fig. 1 where the possible mechanism of their oxidation to the intermediate products is graphically illustrated.

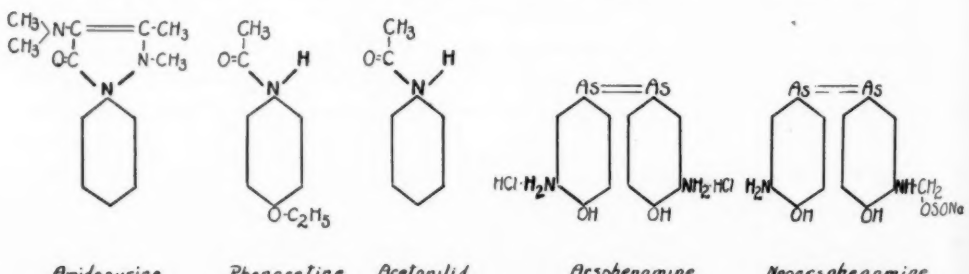
It has been shown by repeated animal experiments that the subcutaneous injection of benzene will produce a depression of the bone marrow with a blood picture in which the leucocyte count falls to 200 or 300 cells with complete absence of granulocytes. It is our opinion that this effect is produced, not by the direct action of benzene upon the bone marrow, but by the action of some of its oxidation products. In support of this contention, rabbits were injected subcutaneously with a mixture of equal parts of benzene and olive oil and the tissues at the site of injection were analyzed eighteen hours later for some of these products. The findings showed benzene to be present only in traces, while phenol and catechol were present in considerable amounts. Analysis of a noninjected control animal showed these products to be absent. Similar results with the additional finding of small amounts of hydroquinone have been reported[42] by Schultzen and Naunyn, Munk, Baumann and Herter, and Nencki and Gaicosa. At the same time bone marrow from the injected animals was analyzed and showed the presence of both phenol and catechol but no trace of benzene. Similar control animals gave entirely negative results. This indicated that benzene as such did not reach the bone marrow but did not exclude further oxidation products of the benzene ring as the causa-

tive factor in the leucocyte depression. The oxidation of hydroquinone or catechol to para-quinone and orthoquinone respectively, can easily be carried out both in vitro and in vivo. In the latter case this reaction is activated by an enzyme, phenolase,[43] which is present in the leucocyte. The following is the progressive oxidation from benzene to quinone:



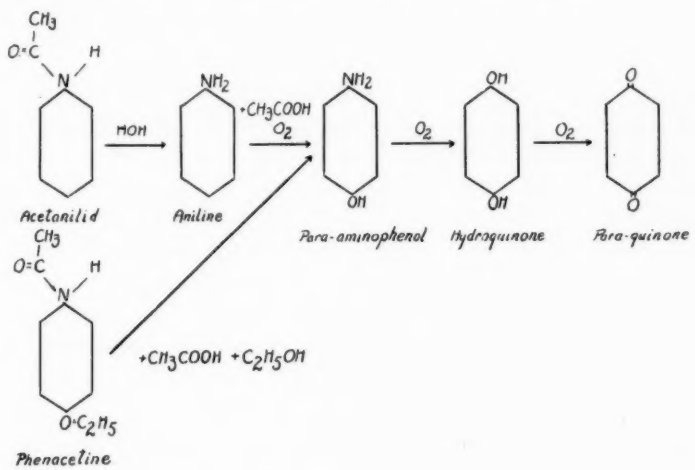
Additional oxidation beyond this point would result in breakage of the ring with the formation of a straight chain carbon compound. It is doubtful that such a compound would have serious toxic action since straight chain carbon compounds are more easily oxidized by the body than the ring containing compounds.

Certain drugs of the coal tar series have been constantly associated with the cases of granulopenia herein reported.[34] It must be noted that these drugs all have as a nucleus the benzene ring with an attached amine ($-NH_2$) group, making them substituted primary amines. This basic structure sets them apart (so far as their reactions are considered) from other coal tar derivatives, such as aspirin, etc., which do not contain the primary amine. For this reason we have arbitrarily designated these compounds as "benzamine drugs" to facilitate reference to them as a group. The drugs contained in this group are amidopyrine, phenacetine, acetanilid, arsphenamine, neoarsphenamine, and those proprietary preparations which contain one or more of the above mentioned. The structural formulas of these drugs are given below with the primary amine nucleus shown in bold face type.



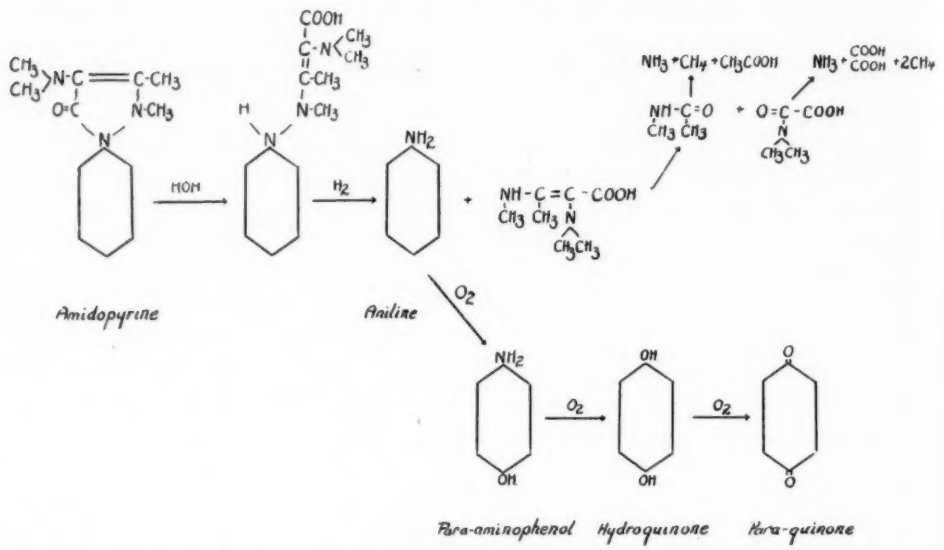
It is well known that the presence of an amine group on the benzene ring greatly increases the ease with which the structure can be oxidized. This property is little influenced by the substitution of additional side chains either upon the ring or upon the amine group itself. With these facts in view, we have worked out the possible oxidation products of these drugs in an attempt to arrive at an end-product common to both benzene and the benzamine compounds. These reactions are logical and sound chemically, but are highly theoretical physiologically, in that they have not been proved so to take place in the animal organism.

The oxidation of acetanilid and phenacetine to para-aminophenol both in vitro and in vivo[44] is well established. The compound has been isolated from the urine[45] and the blood serum[46] of patients to whom these drugs have been administered. The further oxidation of para-aminophenol to quinone[47] is easily carried out in vitro, but as yet, has not been demonstrated in animal metabolism. Hydroquinone is probably formed as an intermediate in this oxidation. These reactions are attended by the splitting off of acetic acid in the case of acetanilid, and of acetic acid and ethyl alcohol in the case of phenacetine. The side chains probably are oxidized further as straight chain carbon compounds. The complete reaction is as follows:



The literature affords little information as to the ultimate fate of amidopyrine in normal or abnormal animal metabolism. Current opinion is summed up in the statement that it is excreted either unchanged[48] or as related substances[49] but no mention is made as to what such related substances might be. Chemically, amidopyrine is a pyrazolon derivative of benzene, or it may be looked upon as a derivative of phenylhydrazine, since this compound is used in its synthesis. The presence of the ketone oxygen on the carbon adjacent to the nitrogen makes this linkage extremely unstable[50] so, it is at this point that the pyrazolon ring would break. This gives rise to a straight chain carbon substitution product of phenylhydrazine. The linkage between the two nitrogens of any phenylhydrazine derivative is easily broken by reduction,[51] regardless of the nature of the substituted side chain. This reaction would

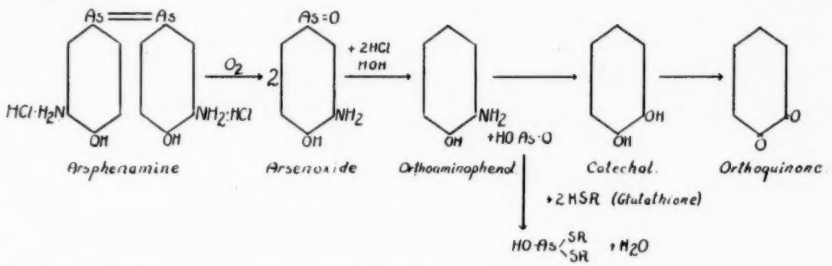
give rise to anilin plus a side chain which may be disposed of as indicated in the following reactions. The anilin thus formed is readily oxidized in vitro to para-aminophenol,[44] hydroquinone and finally to quinone, and therefore might undergo the same reaction in the human body. These reactions are as follows:



Several aspects of the toxicity of arsphenamine have been dealt with by Voegtlin.[52] He explains these either on the physical changes that the drug undergoes when injected into the blood stream, or upon the chemical action of trivalent arsenic with glutathione. It is pointed out that this latter reaction upsets the normal tissue oxidation-reduction equilibrium with a resulting tissue asphyxiation. He mentions the occurrence of "aplastic anemias" in some instances, subsequent to the administration of arsphenamine, but does not explain this marrow depression other than by saying that it may be due to the longer retention of arsenic in the bone marrow than in any other tissue.[53] However, our animal experiments in which trivalent arsenic in the form of Fowler's solution was administered over a period of forty days produced no depression.

This lack of action of arsenic tended to center attention upon the benzamine nucleus of the compound. It is well established that arsphenamine or neoarsphenamine when administered intravenously, is readily decomposed by breakage of the double bond between the arsenic atoms, with the resulting formation of two molecules of arsenoxide.[52] According to Voegtlin, it is this compound which, by virtue of its trivalent arsenic, reacts with the SH of glutathione. He also shows that arsenous acid (HO–As=O) when administered to animals, is more toxic than either arsphenamine or arsenoxide.[54] This would point to the probability that trivalent arsenic may exert its toxic effect equally well regardless of whether or not it is attached to the benzene ring. Therefore, removal of the arsenic from the ring with its hydrolysis to arsenous acid would leave the benzamine group, ortho-aminophenol, free to

be oxidized to catechol, and finally to ortho-quinone. On the other hand, if arsenic is not split from the ring, the hydroxy and the amine groups can be oxidized, without interference, to the quinone form. This chain of reactions is as follows:



From the above reactions, it is obvious that the progressive oxidation of benzene, acetanilid, phenacetine, amidopyrine, and arsphenamine gives rise to several compounds in common. These intermediate and end-products are hydroquinone, catechol, orthoquinone, and para-quinone. It is our belief that one or more of these compounds are responsible for the depressant action of benzene upon the bone marrow, and if so, would point to the benzamine group as also being primary agents in the production of granulopenia.

It must be noted in Table VII that a large percentage of persons in these groups used acetylsalicylic acid in the form of either aspirin or empirin. This compound has as its basis the benzene ring, but no attached amine group. It is well established[55] that this drug is partly hydrolized in the gastrointestinal tract to salicylic acid. The unhydrolized portion is largely excreted as such, while the salicylic acid is excreted mainly unchanged or conjugated with sulphuric and glycuronic acid. A small portion may be oxidized to oxysalicylic acid and hydroquinone. The amount of this latter product would probably be very small since from a chemical standpoint, the absence of the amine group on the ring would be responsible for the slight degree to which this reaction would take place in comparison to the degree of occurrence of this same reaction in the benzamine drugs.

From a study of the various chemical reactions involved, it becomes apparent that the therapeutic effect of most of these drugs is based on one of the oxidation products. It is also true that the bone marrow depressing effect of benzene is due to one of its oxidation products and not to the drug itself. Therefore, it seems probable that bone marrow depression should be produced by the intravenous injection of one or more of these products.

Up to this time we have been able to produce a mild grade of leucopenia in two laboratory animals with the use of hydroquinone as previously reported.[23] At this time we have injected a large series of animals intravenously with hydroquinone, catechol, aniline, para-aminophenol, quinone and phenol, and we have been able to produce mild and severe grades of neutropenia only in an occasional animal and not to such an extent that we are able to state with certainty that we have reproduced the clinical picture. It must be remembered that all laboratory animals do not have inferior bone marrows, and the same situation may exist in the rabbit as we know exists in the

human being, that is, only an occasional human being develops granulopenia, while many thousands of individuals have taken the benzamine drugs indiscriminately and have shown no depression of the bone marrow.

This can well be illustrated in the case of arsphenamine. As shown by the statistics of the Medical Department of the United States Navy[56] nearly 700,000 doses of neoarsphenamine were administered in the eight-year period from 1925 to 1932, inclusive, with untoward reactions totaling 519, or one reaction in 25,000 doses, with only one bone marrow depression to each 90,000 injections. This is equally true of granulopenia, since only 500 cases have been reported in the United States from a population of 120,000,000 people. Therefore, an inferior, weakened bone marrow must be presupposed in the production of this disease. By the use of various oxidation products, we have been able to depress the leucocyte count of rabbits to as low as 1,000 cells per cubic millimeter. Detailed results of these experiments will be presented in a separate communication.

CONCLUSIONS

1. The etiology of granulopenia has not yet been proved.
2. It seems reasonable that a certain percentage of the cases is due to arsphenamine, a certain percentage to gold salts and perhaps other chemicals, leaving, however, a large group of the idiopathic type with an unknown etiology.
3. The disease is more prevalent among physicians and their relatives, nurses, hospital employees and members of the allied professions, than in any other group of people in the United States.
4. It is essentially a disease of the white race.
5. It affects primarily the better class of our people.
6. Its distribution is correlated with the usage of benzamine drugs.
7. A record of 11 cases is presented in which the clinical onset was preceded by prolonged or intensive administration of drugs containing the benzamine group.
8. Statistics are presented to show that this class of drugs has wide usage among members of the medical and allied professions.
9. An hypothesis for its production, based on oxidation reactions of the benzamine drugs, is presented.
10. In the clinical or experimental development of granulopenia, it is necessary to presuppose the existence of a previously weakened, damaged, or idiosyncratic bone marrow which may be congenital or acquired.
11. It is urged that all who have the opportunity to study cases of granulopenia should direct their attention to a careful history of known marrow depressing agents and the usage of benzamine drugs in particular.

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BLOOD IODINE STUDIES*

II. The Normal Iodine Content of Human Blood

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INTRODUCTION

THE constant occurrence of iodine in normal human blood is now firmly established. The ample demonstration of this fact has followed the development of modern micromethods, designed particularly to detect the minute amounts consistently present in clinically obtainable quantities of blood. It is apparent, particularly from the extensive work of Sturm[1] and that of Lunde and his associates,[2] as well as from our own studies,[3] that there is a definite interrelationship between thyroid activity and the blood iodine *niveau*. Furthermore, it is evident that this *niveau* is altered by other physio-

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logic conditions, notably menstruation and pregnancy. There is thus opened up a new approach to studies of thyroid function in health and in disease.

It is evident that it is essential to establish the normal range and average level of the blood iodine in normal individuals, as well as in patients without thyroid disease. Such studies are now available. On the other hand, extensive investigation of the iodine deficiency theory of goiter has led to the conclusion that the iodine intake varies in different regions. Likewise, the manifestations of thyroid disease vary widely in different countries. Too, available iodine, as existing in the food, water, and air, is not the same in various geologic regions. As a consequence it has seemed advisable to make blood iodine determinations of normal individuals in this region; and particularly of our hospital and out-patients without thyroid disease.* We wish to present in this paper these findings, together with a review of the pioneer work on the blood iodine, and a comparison of our results with those of the more recent studies.

HISTORICAL

Baumann's[4] discovery, in 1895, of the presence of considerable iodine within the thyroid gland led to a renewed interest in iodine metabolism as related to thyroid function. Better methods for the determination of iodine were developed, and the blood and other tissues were analyzed for iodine.

The earliest data which we have found for the blood iodine are presented mainly for their historic interest, since they are for the most part at variance with those of the more recent studies. In 1899 Gallard[5] investigated the absorption of iodide by the skin, and its subsequent distribution in the blood and other tissues. He found the iodine content of rabbit blood, after bathing the shaved abdominal skin with sodium iodide solution, to be 635 gamma† per cent. This high value, however, is in accord with other later observations, since it has been shown that the blood iodine rises sharply after iodine administration.[1] In a normal control animal he found 420 gamma per cent. This figure is far too high, in the light of recent studies and of our own work, and indicates contamination, or that the animal had previously received iodine in some form. Gallard used 100 cc. of blood, and a modification of the Rabourdin-Baumann method devised by Bourcet.[6]

Gley and Bourcet,[7] unable to demonstrate iodine in 100 to 200 cc. samples, used a liter of dog's carotid blood to determine the blood iodine. Bourcet's[6] method was followed. They found from 0.013 mg. to 0.112 mg. per liter, or a range of from 1.3 to 11.2 gamma per cent, and an average of 5.5 gamma per cent for the seven dogs. No correlation was observed between the amount of iodine present in the thyroid gland and the blood iodine level. In clotted blood they found the iodine in the serum, and none in the clot. In centrifuged blood the iodine was found only in the plasma. This was not confirmed by Veil and Sturm.[1] Dialysis of the serum or plasma revealed that no iodine

*The determinations reported at this time are all from the Billings Hospital of the University of Chicago.

†A *gamma* is the unit used to designate the minute amounts of iodine present in the blood and tissues. It represents one one-thousandth of a milligram (0.001 mg.). The unit has also been designated a *microgram*.

passed into the dialysate. They consequently concluded that the iodine existed in the plasma in protein combination, and that it was a normal constituent of the blood.

Bourcet[8] investigated the iodine content of other organs and tissues. He found 0.005 mg. of iodine in 200 c.c. of carotid blood pooled from three male rabbits. This is 2.5 gamma per cent. He also found small quantities of iodine in dog's blood. In studying iodine elimination, he examined human menstrual blood. In five individuals this contained from 0.80 to 0.90 mg. of iodine per kilo of blood, or 80 to 90 gamma per cent. In a sixth woman simultaneous epistaxis blood was examined. The menstrual blood contained 94 gamma per cent of iodine, the epistaxis blood contained but 2.1 gamma per cent. He consequently concluded that menstrual blood is an important vehicle of iodine elimination, owing to its high iodine content.

Gley and Bourcet[9] subsequently investigated the effect of exsanguination on the blood iodine of male dogs, using from one-half to one liter of arterial blood. In six normal animals the blood iodine ranged from 2.8 to 9.8 gamma per cent, averaging 7.5 gamma per cent. In two others extreme values were found, 300 gamma per cent in one and less than 1 gamma per cent in another. They found that the blood iodine diminished rapidly after extensive exsanguination, and that after several days, it disappeared completely from the blood. These results are open to serious question.

Blum[10] sought to determine whether the specific "Jodeiweiss" of the thyroid was secreted into the blood stream. He regarded the thyroid as essentially an organ of intraglandular detoxication. In accord with his view the iodine, which Baumann had demonstrated to be present, might be used only in the local economy. With Grützner,[11] a method was devised and tested for the determination, by titration, of organically combined iodine in the presence of organic matter. The "inorganic" iodine was first extracted by acetone. This method was first applied to sheep's blood.

Blum and Grützner[12] subsequently attempted to answer the question of the constant presence of thyroid secretion, as organically combined iodine, in the blood. Using Hunter's[14] method on 50 to 1340 c.c. samples of whole blood, they found from 14 to 140 gamma per cent in five male sheep. However, in four others they failed to detect iodine. They likewise found none in two 125 c.c. samples of goat's blood. Using their own method[11] they detected iodine ("inorganic") but twice in the acetone extract of 6 samples of sheep's blood, and found none at all in the residual coagulum. Likewise no iodine was detected in the blood of 3 dogs, in the blood of 2 men, or in the blood of 2 pregnant women. In the blood of 5 dogs, "jodfrei ernährte," no iodine was found, even though the thyroids contained considerable iodine. They consequently doubted the existence of iodine as a normal constituent of the blood. After giving from 0.5 to 2.0 gm. of sodium iodide to 6 dogs, the blood constantly contained iodine, even up to twenty days after the administration. This was found in the acetone filtrate ("inorganic").

Pathologic bloods were then examined. Unfortunately they were unable to obtain and analyze the blood of patients with exophthalmic goiter, whose blood iodine was subsequently discovered[1] to be high. Among 16 patients

with eclampsia, 10 showed from a trace to 100 gamma per cent of organically combined (coagulum) iodine. Iodine was similarly found in the blood of 2 patients with severe nephritis and in one with convulsions due to a brain tumor. They concluded that iodine in organic combination does not occur in normal blood, and that the "inorganic" iodine occasionally found, comes from the food ingested, or from medication.

Cameron[13] made an extensive investigation of the iodine content of various plant and animal tissues. He used Hunter's method.[14] He found no iodine in dog or rabbit blood, nor in many other tissues. The sample, 0.5 gm., taken for analysis, however, was too small, in view of the minute amount of iodine normally present and the sensitiveness of the method he employed. It would be difficult to demonstrate the presence of iodine in 0.5 gm. of blood even by the most sensitive modern methods.

These pioneer investigations had shown that iodine may be detected by using sufficient amounts of normal mammalian blood and by employing adequate methods. A number of significant questions had thus been raised, among them: Is iodine a constant constituent of mammalian blood? If so, in what combination does it exist—organic, inorganic, or both? What is its relation to the thyroid gland, and to the thyroid iodine? What is its relation to thyroid disease? The answer to these, as well as further progress, was dependent upon the development and application of more sensitive methods for the biochemical determination of iodine.

"*The first modern figure for blood iodine,*" as Orr and Leitch[15] are pleased to term it, is that of Kendall and Richardson[16] for ox blood, 13 gamma per cent.* Kendall's method[17] was used on 100 c.c. samples. In twenty-three analyses, the level ranged from 10 to 17 gamma per cent, averaging 13 gamma per cent. In sixteen subsequent analyses, known amounts of iodine were added to the water solution after charring and fusing the blood. The amount added was subsequently subtracted from that determined. The range in these was from 4 to 21 gamma per cent. The average, however, was the same, 13 gamma per cent. When known amounts of iodine were added to whole blood, from 2.2 to 3.0 per cent was not recovered by the analysis. The lower limit of detectable iodine by Kendall's method is about 5 gamma (0.005 mg.).

Gley and Cheymol, observing that the goat thyroid contained considerable iodine,[19] examined the efferent blood from the thyroid veins of eight female goats.[18] In this manner they attempted to demonstrate the passage into the blood of the iodine-containing thyroid secretion. Kendall's method was used on 100 c.c. samples. The carotid blood was found to contain from 4.3 to 26 gamma per cent, averaging 12.3 gamma per cent. Efferent thyroid blood, however, was found to contain from 5 to 50 gamma per cent, averaging 20.8 gamma per cent. They consequently concluded that they had demonstrated the passage of the internal secretion of the thyroid into the blood stream. No correlation was observed between the varying iodine content of the thyroid gland and that of the efferent blood.

*This is variously quoted in the literature as for human or dog blood. Kendall[21] used ox blood for the determinations. A large series of determinations were also made on human and dog blood. The values were all within a narrow range.

Cheymol and Gley,[20] using Kendall's method, found 13 gamma per cent of iodine in the pooled blood from 5 male guinea pigs, and 11 gamma per cent in the pooled blood of 5 females. In dog's carotid blood they found 9.6 gamma per cent, and in the blood of the female goat 12.4 gamma per cent. Samples of 100 c.c. were used for analysis.

RECENT INVESTIGATIONS

During the first decade of the twentieth century advancing goiter research had created a demand for an adequate method for the determination of minute amounts of iodine. In 1922 the Swiss Goiter Commission consequently requested von Fellenberg, of the Government Bureau of Hygiene, to develop such a method. He devised, perfected, and eventually synthesized the known existing quantitative procedures into a true micromethod for the determination of iodine.[22] The lower limit of detectable iodine by this method is about one ten-thousandth of a milligram (0.0001 mg.). Two of his pupils, Sturm and Lunde, soon applied his method to the study of the blood iodine in thyroid disease.

The successful application of the principles developed in von Fellenberg's method has led to such extensive developments that from this point we must limit ourselves to the discussion of normal human blood iodine values. Since determinations of the blood iodine on hospital patients have been used by some investigators as normal, we are including them in this report. However, we are omitting all blood iodine reports on goitrous patients, on patients with dietary deficiency diseases, on patients with acute inflammations, and on those with leucemia, as well as blood iodine determinations made on women during early menstruation, pregnancy, and lactation.

Alexander Sturm learned von Fellenberg's method at Berne. Returning to Veil's Clinic in Munich, he investigated extensively the blood iodine in normal individuals, in patients with thyroid disease, and in those with various other diseases. His study[1] presented, in 1925, the first comprehensive data of normal human blood iodine values. In a series of 36 individuals, studied in the late summer and fall, the blood iodine averaged 12.8 gamma per cent. In 24 normal individuals investigated during late fall and winter the average was lower, 8.3 gamma per cent. The iodine occurred in similar concentration in the plasma and in the corpuscles. When the blood proteins were precipitated by alcohol, about 35 per cent of the iodine was recovered in the alcoholic filtrate. The remaining 65 per cent was found in the precipitate. From Jena, in 1928, Sturm[23] reported a series of blood iodine determinations made on 10 men. These revealed high values in May and July, of 15.0 and 14.1 gamma per cent, respectively. The lowest level, found in December, was 9.9 gamma per cent.

Maurer and Diez,[24] of Munich, investigated the blood iodine during menstruation and pregnancy. During this study they determined the blood iodine of 10 women during the intermenstrual period. These determinations were made during the winter. They ranged from 4.2 to 15.0, averaging 9.2 gamma per cent. Later, Jahn and Kesselkaul,[25] also of Munich, studied the variation of the blood iodine during the menstrual cycle. They reported that 17 normal

women had intermenstrual blood iodine values ranging from 7.2 to 12.0 gamma per cent, and averaging 9.5.

Jansen[26] reported an average of 12 to 14 gamma per cent in normal individuals at Bonn.

In the study which DeQuervain and Smith[27] made at Berne, on the blood iodine of cretins and of patients with nontoxic nodular goiter, they included a series of determinations made on five normal individuals during the winter. These determinations ranged from 11.1 to 14.6 gamma per cent, with an average of 13.1.

Lunde, Closs and Pedersen,[2] reported 11 to 16 gamma per cent as the normal blood iodine level occurring in Oslo. For fractionating the blood iodine, Lunde devised a method of alcohol extraction. This differs from Sturm's[1] method of alcohol precipitation. By Lunde's method only 1 to 4 gamma per cent was found in the alcohol insoluble fraction. Work from Lunde's laboratory indicates that the alcohol insoluble fraction is of particular significance in the pathologic physiology of toxic goiter.

At Aberdeen, Orr and Leitch[15] determined the blood iodine by the Leitch and Henderson method. They found in 16 normal individuals, examined in the spring, an average of 6 gamma per cent, while that of 28 individuals, examined in the fall, averaged 8.4.

In 1929 Eisler and Schittenhelm,[28] using the von Fellenberg method, reported from 10 to 13 gamma per cent as the normal blood iodine level for Kiel. The same investigators in 1932,[29] reporting seventy-one blood iodine determinations, gave from 7 to 9 as the range for winter and from 9 to 12 as the range for the summer.

Fowweather,[30] while studying the blood iodine in patients with cancer, estimated the blood iodine in twelve normal individuals. He found a range of from 3.6 to 5.1 gamma per cent, with an average of 4.3. This investigation was made at Leeds, using the Leitch and Henderson method.

Baldauf and Pincussen,[31] who studied the interrelationship between the bromine and iodine concentration of the blood, reported an average of 10.9 gamma per cent for a series of blood iodine determinations made on normal individuals in Berlin. Scheringer,[32] also in Berlin, in a series of 9 individuals studied between January and July, found from 6 to 15 gamma per cent, with an average of 8 gamma per cent. The following year he reported[33] another group of 8 individuals with a range of 10 to 13. Twelve of Scheringer's 17 subjects were women and the intermenstrual values were taken as the normal for them.

In 1930 Turner,[34] of Detroit, reported 9 cases on which he found a range of from 9 to 15, and an average of 11 gamma per cent. He used his own method for the determination of the blood iodine.

Schneider and Widmann,[35] of Freiburg, used von Fellenberg's method on 15 patients, 5 normal subjects and 10 nongoitrous patients. They found the blood iodine to range from 8.2 to 17.5 gamma per cent, with an average of 12.5. A year later Widmann[36] used his own modification of the Schwaibold

closed oxidation procedure for blood iodine on 6 nongoitrous patients. He found blood iodine values ranging from 27 to 42 gamma per cent, with an average of 32. This modification needs further investigation to rule out the possibility of other oxidizing substances than iodine containing radicles being carried over in the oxidation process, and later reacting with the added potassium iodide.

Biilmann[37] found a range of 8 to 18 gamma per cent in 10 individuals. The patient on whom Bøe and Elmer[38] studied the effect of intravenous thyroxin administration had a normal blood iodine of 12 gamma per cent.

An extensive study of the blood iodine fractions in normal subjects was reported from Paris by Nitzescu and Binder.[39] Their investigations were made on a group of healthy male subjects ranging in age from twenty to thirty years. The lowest average blood iodine, 8.7 gamma per cent, was found in 47 cases studied in February. The highest averages were found in June, 12.7 for 16 cases, and in July, 12.9 for 11 cases. The average inorganic fraction ranged from 2.2 gamma per cent in January to 5.1 gamma per cent in June, while the organic fraction was lowest in February at 6.1 and highest in July at 8.1 gamma per cent. There was thus a greater variation in the inorganic than in the organic fraction. The separation was carried out according to Lunde's procedure. In this determination the "organic" fraction contains all of the alcohol insoluble fraction plus the "organic" portion of the alcohol soluble fraction.

While studying the relationship of the iodine concentration in the blood to that in the cerebrospinal fluid, Hahn and Schurmeyer[40] found in 17 individuals a range of 7 to 14 gamma per cent, with an average of 10.6.

Dodds, Lawson and Robertson,[41] working in London and using Lunde's method, found in 7 normal subjects under forty years of age, an average of 10.2 gamma per cent with a range of from 7.8 to 17.6. The alcohol soluble fraction in the 7 varied from 4.9 to 11.9 gamma per cent, with an average of 7.7. The alcohol insoluble fraction in 16 cases varied from 2.4 to 5.8 gamma per cent, with an average of 4.

OBSERVATIONS

The blood iodine determinations here reported were all made by Davis'[42] method. This is a development of the von Fellenberg procedure. The results obtained are best presented in tabular form (Tables I, II and III).

In Table I is presented a series of 8 determinations made on 6 normal members of the Billings Hospital staff. The determinations on the women were all made during the intermenstrual period. These individuals were all taking the usual hospital diet, which contains no iodized salt. The range of the 8 determinations is between 8.9 and 13.8, and the average 12 gamma per cent.

In Table II is presented a series of 13 determinations made on 10 hospital patients. The diagnoses are given. None of these patients revealed evidence of thyroid disease. All were under careful observation, and on controlled diets which contained no iodized salt. The age range is wide, from thirteen

to seventy-eight years. The blood iodine ranges from 8.5 to 14.4, averaging 12.3 gamma per cent. In accord with Fowweather[30] we observe that the presence of cancer has no obvious effect upon the level of the blood iodine.

TABLE I

BLOOD IODINE IN NORMAL INDIVIDUALS ON USUAL HOSPITAL DIET

CASE	AGE	SEX	BLOOD IODINE GAMMA PER CENT	DATE
Technician	24	M	11.8	9/ 9/30
Chemist	25	F	13.3	10/ 6/31
			13.8	1/ 8/32
Nurse	25	F	8.9	11/16/31
Nurse	28	F	13.8	11/ 5/31
			9.3	11/18/31
Nurse	29	F	12.3	4/20/31
Student	30	F	13.1	1/10/32

TABLE II

BLOOD IODINE IN HOSPITAL PATIENTS WITHOUT THYROID DISEASE

CASE	AGE	SEX	DIAGNOSIS	BLOOD IODINE GAMMA PER CENT	DATE
36112	13	M	Obesity	10.4	4/ 6/31
22859	20	M	T. B. spine	13.9	7/ 2/30
				13.5	8/ 5/30
24761	27	F	Paronychia	8.5	7/ 7/30
17938	33	F	Chronic osteomyelitis	12.0	8/ 4/30
				14.1	8/18/30
32540	41	M	Anal ulcer	12.5	1/ 8/31
22940	52	M	Fracture tibia	13.5	7/ 2/30
				11.8	8/ 5/30
40972	57	F	Melanosarcoma	11.2	7/ 3/31
41561	57	M	Carotid body Ca.	14.4	7/ 7/31
41128	68	M	Ca. of mouth	14.4	7/ 9/31
25863	78	M	Ca. of pharynx	9.9	8/ 1/30

TABLE III

BLOOD IODINE IN OUT-PATIENTS WITHOUT THYROID DISEASE

CASE	AGE	SEX	DIAGNOSIS	BLOOD IODINE GAMMA PER CENT	DATE
27006	6	M	Normal	12.7	8/14/30
28169	16	F	Infantile paralysis	16.2	9/15/30
30383	23	F	Retroverted uterus	12.7	3/12/31
24761	27	F	Paronychia	11.2	8/ 7/30
28425	29	F	T. B. cervical lymphadenitis	8.5	9/19/30
28499	30	F	T. B. cervical lymphadenitis	9.2	9/22/30
28500	31	M	Furuncle of arm	10.6	9/22/30
24531	31	F	"Neurasthenia"	9.3	7/14/30
27651	41	M	Chronic spondylitis	9.5	9/ 4/30
30545	43	M	Chronic osteomyelitis	12.7	3/16/31
				11.8	3/23/31
31827	46	F	Cervical lymphoblastoma	12.7	3/23/31
27653	48	M	Tibial tuberculid	11.4	9/ 4/30

In Table III is presented a series of 13 determinations made upon 12 ambulatory patients visiting the out-patient clinic. All presented no evidence of thyroid disease. None was using iodized salt. The range is from 8.5 to 16.2 and the average is 11.4 gamma per cent. The range is thus somewhat

TABLE IV

NORMAL HUMAN BLOOD IODINE

OBSERVER	DATE	LOCALITY	METHOD	RANGE	AVERAGE	NUMBER OF CASES	SEASON
Veil and Sturm[1]	1925	Munich	v. F.*		12.8	36	Late Summer and Fall
					8.3	24	Late Fall and Winter
Maurer and Diez[24]	1927	Munich	v. F.	4.2-15	9.2	10	Winter
Sturm[23]	1928	Jena	v. F.		10.4	10	November
					9.9	10	December
					11.0	10	January
					15.0	10	May
					14.1	10	July
					12.1	10	September
					10.4	10	November
Jansen[26]	1928	Munich	v. F.	12-14			
DeQuervain and Smith[27]	1928	Berne	v. F.	11.1-14.6	13.1	5	Winter
Jahn and Kesselkaul[25]	1928	Munich	v. F.	7.2-12	9.5	17	
Lunde, Closs and Pedersen[2]	1929	Oslo	v. F.	11-16			
Orr and Leitch[15]	1929	Aberdeen	L. & H.		6.0	16	Spring
					8.4	28	Fall
Eisler and Schittenhelm[28]	1929	Kiel	v. F.	10-13			
Fowweather[30]	1930	Leeds	L. & H.	3.6-5.1	4.3	12	

TABLE IV—CONT'D

OBSERVER	DATE	LOCALITY	METHOD	RANGE	AVERAGE	NUMBER OF CASES	SEASON
Baldauf and Pincussen[31]	1930	Berlin	v. F.		10.9		
Scheringer[32]	1930	Berlin	v. F.	6-15	8.0	17	Winter
Turner[34]	1930	Detroit	Own	9-15	11.0	9	
Scheringer[33]	1931	Berlin	v. F.	10-13		8	Winter
Schneider and Widmann[35]	1931	Freiburg	v. F.	8.2-17	12.5	15	Fall
Biilmann[37]	1931			8-18			
Bøe and Elmer[38]	1931	Berlin			12.0	1	
Nitzescu and Binder[39]	1931	Paris	v. F.		8.4 8.7 9.4 12.7 12.9 11.4 11.3	19 47 41 16 11 29 10	January February March June July September December
Schittenhelm and Eisler[29]	1932	Kiel	v. F.	7-9 9-12		71	Winter Summer
Widmann[36]	1932	Freiburg	Own	27-42	32.0	6	
Hahn and Schurmeyer[40]	1932		v. F.	7-14	10.6	17	
Dodds, Lawson and Robertson[41]	1932	London	v. F.	7.8-17.6	10.2	7	
Davis, Curtis, and Cole	1934	Chicago	v. F.	8.5-16.2	11.9	28	

*v. F. = von Fellenberg.
L. & H. = Leitch and Henderson.

greater. This may be due to the fact that there was no dietary control and that certain of the bloods were not drawn in the postabsorptive state.

In all, 34 determinations made on 28 individuals without evidence of thyroid disease revealed a range of from 8.5 to 16.2 and an average of 11.9 gamma per cent. It would thus appear that about 12 gamma per cent is the normal average blood iodine level in Chicago.

The 18 determinations made upon women ranged from 8.5 to 16.2, averaging 11.6 gamma per cent. The 16 determinations made upon men ranged from 9.5 to 14.4, averaging 12.2 gamma per cent. The greater range of the female levels is presumably to be associated with the cyclic variations in the blood iodine due to menstruation. The male average level is slightly higher.

DISCUSSION

The results of available studies upon the normal blood iodine are presented in Table IV. Omitting the very low results of Fowweather[30] and the very high results of Widmann,[36] the average normal levels for the blood iodine vary from 6.0 to 13.1 gamma per cent. Since iodine loss is the most important single factor in making the determinations by the open oxidation methods, we are inclined to regard those results at the upper range as more nearly the true condition. A study of the table would lead us to conclude that the blood iodine in normal individuals, or in patients with normal thyroid function, is only influenced slightly, if at all, by geographic location.

The seasonal variation of the blood iodine is of interest. It corresponds to that observed in the thyroid gland by Seidell and Fenger.[43] A tabulation of available data readily shows a confirmation of the results first announced by Veil and Sturm,[1] that the highest values are obtained in the summer months and the lowest in the winter.

The fractionation of the blood iodine into an alcohol or acetone soluble, and an insoluble fraction is doubtless of significance. We have not yet attempted this procedure. It is apparent that the alcohol insoluble fraction is approximately the same when the determination is consistently made by the same method. It varies greatly, however, with the mode of extraction. The work of Lunde and his associates on the blood iodine in hyperthyroidism points to the significance of the alcohol insoluble fraction. *Presumably* it is the thyroid hormone. The "inorganic" iodine may come from the diet or from catabolism of the "organic" iodine compounds.

SUMMARY

Iodine is constantly present in the human blood. The average level is about 12 gamma per cent. Development of our knowledge regarding this fact followed the institution of adequate micromethods for the determination of the minute amounts normally present. The blood iodine varies seasonally, being higher during the summer and lower during the winter months. The blood iodine may be separated into "organic" and "inorganic" fractions. Determinations of the blood iodine open up a new approach to the study of thyroid function in health and in disease.

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THE SEDIMENTATION RATE AND POLYMORPHONUCLEAR COUNT IN RHEUMATOID AND MIXED ARTHRITIS*

Their Value as an Index to Activity

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THE sedimentation rate and the polymorphonuclear count (hereafter referred to as the nuclear count) have been applied in the study of a number of different diseases, but it has been only during the past few years that they have been used in the study of arthritis. A number of papers have been published on the value of the sedimentation rate in arthritis but comparatively few have appeared on the nuclear count.

Various investigators[1, 2, 3, 4] have stated that the sedimentation rate is greatly increased in rheumatoid arthritis while in osteoarthritis the rate is usually normal or only slightly increased. It has been used mostly as an aid in differentiating these two types of arthritis. It has been reported[3] that there is a close relationship between the sedimentation rate and the severity of the infection; that remissions are associated with a decreased rate and exacerbations with an increased rate. Some[5, 6] have suggested that this test may be of assistance in following the progress of the patient's illness. They have not, however, reported the frequency with which these changes occur. Kahlmeter[1] suggested the use of the sedimentation rate as an index of activity, a knowledge of which he believes to be essential for the proper use of physiotherapy. Forestier[4] has shown that as the patient improves, the sedimentation rate returns to normal.

The value of the nuclear count is based on the observation that an active infection is accompanied by an increase in the number of young polymor-

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phonuclear neutrophiles in the blood. An increase in the number of these cells is termed "shift to the left." Only recently has this principle been used in the study of arthritis. Schilling[7] stated that in rheumatoid arthritis only slight changes are present in the hemogram, but other investigators using the Schilling hemogram have reported more conclusive findings. Cecil[8] reported a shift to the left in 52 per cent of 23 cases of rheumatoid arthritis and that Resnikoff found a shift to the left in 67 per cent of 28 cases. Eaton[9] in his study of 250 cases of chronic nontuberculous arthritis found a shift to the left in 90.4 per cent. Gerard and Boerner[10] reported two cases, and Piney[11] one, of arthritis with a shift to the left. Recently, Steinbrocker and Hartung,[12] using the filament nonfilament count, a modification of the Schilling hemogram advocated by Farley, St. Clair, and Reisinger, reported finding what is the equivalent of a shift to the left in 100 per cent of 50 cases of rheumatoid arthritis.

The present study was undertaken to determine whether exacerbations and the periods of improvement in patients with rheumatoid and mixed arthritis are accompanied by definite changes in the sedimentation rate and the nuclear count and the relationship between the two tests. We chose for our study a group of patients who attended the clinic regularly. At each visit a detailed record of both the subjective complaints and the objective findings was made. The presence of any intercurrent infections was also noted. Whenever there was a change in the condition of the patient, the sedimentation rate and nuclear count were determined. In a number of instances these determinations were made when there was no change in the patient's condition. They were always made at approximately the same time of the day. Westergren's[13] modification of Fahraeus' technic was used for the sedimentation, and a rate of 12 mm. or less per hour was considered normal. In making the nuclear counts, the technic of Cooke and Ponder[14] was used. Farley, St. Clair, and Reisinger,[15] in reviewing the work of Arneth, Schilling, and Cooke and Ponder, state that the classification of Arneth is too complicated for practical use, and that the method of Schilling lacks the clear-cut differentiation which can be obtained by following Cooke's criterion. This criterion is as follows: "If there is any band of nuclear material except a fine filament of nuclear material connecting the different parts of a nucleus, that nucleus for the purpose of the count cannot be said to be divided." With this as a basis, the polymorphonuclear neutrophils were divided into five groups according to the number of segments. In determining the presence or absence of a shift to the left, Cooke and Ponder considered as an average normal count, 12 Type I, 25 Type II, 44 Type III, 15 Type IV, and 4 Type V cells per 100 polymorphonuclear neutrophils.

In the early part of this work we failed to realize the importance of intercurrent infections, but as the work proceeded we excluded all those tests which were made when the patient was suffering from such infections. Determinations were made in 50 cases each of rheumatoid and mixed arthritis. A total of 262 sedimentation tests were done in the former and 234 in the latter group. Of the patients with rheumatoid arthritis 89 per cent had a

sedimentation rate above normal. The average rate was 36 mm. per hour. In 53 per cent of the tests the values were above 30 mm. per hour. Of the 234 determinations in mixed arthritis, 64 per cent were above normal. The average rate in this group was 18 mm. per hour with only 15 per cent of the cases showing values above 30.

Of 258 nuclear counts made on 50 patients with rheumatoid arthritis, 68 per cent showed a shift to the left. In 23 per cent the counts were within normal limits and in 9 per cent there was a slight shift to the right. In the cases of mixed arthritis, 244 counts were made, of which 57 per cent showed a shift to the left, 36 per cent presented normal counts and 7 per cent showed a shift to the right.

In 210 instances in the rheumatoid group the sedimentation rate and the nuclear count were compared. In 77 per cent a shift to the left was associated with a high sedimentation rate. In 10 per cent there was a normal sedimentation rate and either a shift to the right or a normal count. In 6.5 per cent there was a normal count or a shift to the right accompanied by a high sedimentation rate, and in 6.5 per cent there was a shift to the left with a normal sedimentation rate. In the series of mixed arthritis, 190 comparisons were made. Of these, 58 per cent presented both a shift to the left and a high sedimentation rate, while 14 per cent showed a normal sedimentation rate and either a normal count or a shift to the right. In 23 per cent of the cases in this group, a shift to the left was accompanied by a normal sedimentation rate, and in 6 per cent there was a high sedimentation rate contrasted with either a normal count or a shift to the right (Table I).

TABLE I

CORRELATION OF THE SEDIMENTATION RATE AND THE NUCLEAR COUNT

	RHEUMATOID PER CENT	MIXED PER CENT
High sedimentation rate Shift to the left	77.0	58
Normal sedimentation rate Normal nuclear count	10.0	14
High sedimentation rate Normal nuclear count	6.5	6
Normal sedimentation rate Shift to the left	6.5	22

In 274 instances the sedimentation rate, the nuclear count and the clinical condition of the patients were simultaneously recorded in order to study the value of these tests as an index to activity. In this group no differentiation was made between the rheumatoid and mixed cases. Of the total number, 119 were made when there was improvement in the clinical symptoms, 56 when they were worse, and 99 when they were unchanged. Of the 119 made during clinical improvement, 74 per cent showed a definite reduction in both the sedimentation rate and the degree of the shift to the left; 6 per cent showed a slight increase in the sedimentation rate and a further shift to the left; 9 per cent showed no change in either the sedimentation rate or the nuclear

count; 6 per cent showed no change in the nuclear count but a reduction in the sedimentation rate; 3 per cent showed an approach to normal in the nuclear count but no change in the sedimentation rate; 2 per cent showed an approach to normal in the nuclear count and a slight increase in the sedimentation rate. Of the determinations made when there was an exacerbation of the clinical symptoms, 65 per cent showed an increase both in the sedimentation rate and in the degree of the shift to the left, while 14 per cent showed a slight reduction in both. In 14 per cent there was an increase in the sedimentation rate but a decreased shift to the left; in 7 per cent there was no change in the nuclear count but a slight reduction in the sedimentation rate. Where the clinical condition was unchanged, 49 per cent showed no change in either test. In 24 per cent there was a slight reduction in the sedimentation rate and in the shift to the left; 4 per cent showed a slight reduction in the sedimentation rate and an increased shift to the left; 6 per cent showed a slight increase in the sedimentation rate and in the shift to the left; 3 per cent presented a slight increase in the sedimentation rate but no change in the nuclear count; 3 per cent showed no change in the sedimentation rate but a decreased shift to the left; 3 per cent gave an increased sedimentation rate and a decreased shift to the left; 3 per cent gave no change in the sedimentation rate but an increased shift to the left, and 5 per cent gave a decreased sedimentation rate and no change in the nuclear count. (Table II.)

TABLE II

CHANGES IN THE SEDIMENTATION RATE AND THE NUCLEAR COUNT CORRELATED WITH CHANGES IN THE CLINICAL CONDITION

SEDIMENTATION RATES AND NUCLEAR COUNTS	CLINICAL CONDITION		
	IMPROVED	WORSE	UNCHANGED
	PER CENT	PER CENT	PER CENT
1. Sedimentation rate decreased Shift to the left decreased	74	14	24
2. Sedimentation rate increased Shift to the left increased	6	65	6
3. Sedimentation rate unchanged Nuclear count unchanged	9		49
4. Sedimentation rate decreased Nuclear count unchanged	6	7	5
5. Sedimentation rate unchanged Shift to the left decreased	3		3
6. Sedimentation rate increased Shift to the left decreased	2	14	3
7. Sedimentation rate decreased Shift to the left increased			4
8. Sedimentation rate increased Nuclear count unchanged			3
9. Sedimentation rate unchanged Shift to the left increased			3
10. Sedimentation rate alone increased	8	79	12
11. Nuclear count alone increased	6	65	13
12. Sedimentation rate alone unchanged	12		55
13. Nuclear count unchanged	15	7	57
14. Sedimentation rate alone decreased	80	21	33
15. Nuclear count alone decreased	79	28	30

DISCUSSION

Although several articles have been published on both the sedimentation rate and the nuclear count in arthritis, there have been no reports of a correlation of the two tests and their value as indicators of activity. They have been used mainly as an aid in the differential diagnosis between rheumatoid and osteoarthritis. Our study indicates that these tests are of considerable value as an index of the activity of the arthritic process in patients with rheumatoid and mixed arthritis and as such are efficient aids in judging the progress of the patient. When the clinical condition is improved, there is usually a decrease in the sedimentation rate and a lessened shift to the left. Conversely, an increase in the severity of the arthritic process is associated with a rise in the sedimentation rate and a further shift to the left. The degree of change in these tests is fairly proportionate to the degree of change in the clinical condition. On numerous occasions we noted that intercurrent infections, such as colds and sore throats, adversely affected the sedimentation rate and nuclear count, and they, therefore, must be excluded before attributing changes in the tests to changes in the course of the disease. In the absence of any infection, however, the sedimentation rate and nuclear count tend to return to normal when the clinical symptoms have subsided. We have also observed that a hidden focus of infection will frequently give a persistent, high sedimentation rate and a shift to the left.

CONCLUSIONS

1. In the majority of cases of rheumatoid and mixed arthritis the sedimentation rate and the nuclear count serve as an index of activity, and they may, therefore, be used as a means of determining the progress of these diseases.

2. The degree of change in the clinical symptoms is reflected in parallel changes in the sedimentation rate and nuclear count.

3. Intercurrent and focal infections adversely affect the sedimentation rate and nuclear count, and their presence must be excluded before changes in these tests may be attributed to variations in the activity of the arthritic process.

4. The variations in the sedimentation rate and the nuclear count do not correspond 100 per cent to the variations in the arthritic state, and therefore, neither alone is finally diagnostic; and knowledge of both the sedimentation rate and the nuclear count together should increase the accuracy of the interpretations.

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THE BACTERICIDAL AND FUNGICIDAL ACTION OF HOMOLOGOUS HALOGEN PHENOL DERIVATIVES AND ITS "QUASI-SPECIFIC" CHARACTER*

I. Derivatives of Parachlorophenol

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INTRODUCTION

In TWO recent papers[1] we described the preparation of a number of halogen phenol derivatives with bactericidal properties, giving also a survey of published work which preceded our investigations in this field. While all the derivatives studied by us were found to be germicidal toward a number of microorganisms among which were several resistant pathogenic bacteria and fungi, some of them were shown to possess this capacity to a remarkably high degree. In following up the relationship between the chemical structure and the bactericidal action of these compounds we observed a number of regularities, viz.:

1. Halogen substitution intensifies the microbicidal potency of phenol derivatives, the presence of halogen in the para-position to the hydroxyl group being more effective in this respect than in the ortho-position.

2. Introduction of aliphatic or aromatic groups into the nucleus of halogen phenols increases the bactericidal potency (up to certain limits), this increase depending, in the case of alkyl substitution, upon the number of carbon atoms present in the substituting group or groups.

3. As a rule the intensifying effect upon the bactericidal potency of a normal aliphatic chain with a given number of carbon atoms is greater than

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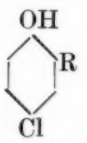
that of a branched chain, or of two or more alkyl groups with the same total number of carbon atoms.

4. Orthoalkyl derivatives of parachlorophenol are more actively germicidal than paraalkyl derivatives of orthochlorophenol.

5. In the case of the higher homologues the germicidal action manifests a "quasi-specific" character in that beginning with a definite point (which is different in the case of the various test organisms), with the further increase of the weight of the substituting groups, the germicidal capacity drops to almost total inactivity with respect to certain microorganisms *(Eberthella typhi, Eberthella paradysenteriae)*, while rising to comparatively enormous values with respect to others *(Staphylococcus aureus,* streptococcus, *Mycobacterium smegmatis)*.

It is the object of further work to extend the scope of our inquiry into the bacteriologic and pharmacologic phases of this problem, with the ultimate view of ascertaining the possible utility of these compounds for therapeutic purposes. The present paper furnishes the data obtained thus far with compounds derived from parachlorophenol; these compounds may be represented by the general formula



where R is an aliphatic or aromatic substituent.

The following microorganisms were used in this investigation: *Eberthella typhi, Escherichia coli, Eberthella paradysenteriae* (Flexner), *Salmonella schottmülleri (B. paratyphosus B), Staphylococcus pyogenes aureus,* streptococcus (hemolytic strain), *Micrococcus catarrhalis, Mycobacterium smegmatis, Mycobacterium tuberculosis (hom.), Mycobacterium tuberculosis (avium), Mycobacterium leprae (mur.), Mycobacterium leprae (hom.), Trichophyton rosaceum, Monilia albicans,* and *Achorion schönleinii.*

MONOALKYL DERIVATIVES

Tables I to IV illustrate the microbicidal action of the series of monoalkyl derivatives of parachlorophenol in relation to their chemical constitution, and more particularly to the increase in length (or weight) of the substituting side chain. Wherever possible, the minimum germicidal concentrations have been determined and the "phenol coefficients" calculated. Table I gives the results obtained with four gram-negative organisms of the typhoid colon group, in Table II the test organisms are (gram-positive and gram-negative) cocci, in Table III they are bacteria of the acid-fast group, while Table IV refers to three pathogenic fungi.*

When considered individually, certain among the several compounds reveal a bactericidal effect of considerable intensity. However, in addition there is also unmistakable evidence of a parallelism in both a qualitative and

*The figures given in parentheses are of relative significance only, as alcohol in excess of 10 per cent but below 20 per cent had to be used in order to produce the desired concentrations (owing to the very limited solubility of the respective compounds).

TABLE I

THE BACTERICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL UPON ORGANISMS OF THE TYPHOID-COLON GROUP. MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	EBERTHELLA TYPHI		EBERTHELLA PARADYSENTERIAE		SALMONELLA SCHOTTMÜLLERI (B. PARATYPHOSUS B.)		ESCHERICHIA COLI	
	I	II	I	II	I	II	I	II
p-Chlorophenol	1: 650	4.3	1: 700	4.7	1: 600	4.3	1: 600	4.6
Alkyl Radical								
Methyl	1: 2,000	12.5	1: 2,000	14.3	1: 1,800	12.9	1: 2,000	14.3
Ethyl	1: 4,000	28.6	1: 4,500	32.1	1: 4,000	28.6	1: 3,500	26.9
n-Propyl	1:14,000	93.3	1:14,000	100.0	1:10,000	714.0	1:12,000	85.7
n-Butyl	1:22,500	141.0	1:25,000	167.0	1:16,000	114.0	1:16,000	114.0
n-Amyl	1:25,000	156.0	1:30,000	200.0	1:14,000	100.0	1:20,000	154.0
Sec. Amyl	1: 7,000	46.7	1:12,000	80.0	1: 6,000	42.9	1: 5,000	41.7
n-Hexyl	(1: 3,250)	(23.2)	1:50,000	333.0	(1: 3,000)	(21.4)	(1: 5,000)	(35.7)
Cyclohexyl	>1: 4,000	<26.7	1:12,000	75.0	>1: 2,000	<14.3	>1: 2,000	<14.3
n-Heptyl	(1: 3,000)	(20.0)	(1: 3,000)	(20.0)	(1: 2,000)	(14.3)	(1: 2,000)	(14.3)
Phenol (control)	1:140-150	1.0	1:140-160	1.0	1:140-150	1.0	1:140-150	1.0

quantitative respect in the reaction toward the homologues of this series, of the microorganisms in Table I on one hand, and of those in the remaining three tables on the other.

Thus, in Table I the bactericidal efficacy rises with the increasing weight of the substituting side chain reaching a maximum with the n-butyl derivative in the case of *Salmonella schottmülleri*, and with the n-amyl derivative in the cases of *Eberthella typhi* and *Escherichia coli*, while the n-hexyl derivative is the most effective against *Eberthella paradysenteriae*. After reaching the maximum, one observes a rather abrupt drop of the bactericidal potency with the further increase of the weight of the substituents.

In agreement with observations made with other classes of phenol derivatives, here, too,[2, 3, 4] substitution by branched or cycloalkyl groups leads to compounds of lower bactericidal potency than that of the normal alkyl derivatives with the same number of carbon atoms in the substituting group.

An entirely different picture is furnished by Table II. Here, too, the coccidal potency increases with the increasing weight of the substituting radical, but its range extends beyond the turning points of the preceding table.

TABLE II

THE GERMICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL UPON PATHOGENIC COCCI.

MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	STAPHYLOCOCCUS AUREUS		STREPTOCOCCUS (HEMOL. STRAIN)		MICROCOCCUS CATARRHALIS	
	I	II	I	II	I	II
p-Chlorophenol	1: 300	4.3	1: 400	4.4	1: 600	4.0
Alkyl Radical						
Methyl	1: 1,000	12.5	1: 1,000	11.1	1: 1,600	10.7
Ethyl	1: 2,750	34.4	1: 2,500	31.3	1: 4,000	26.7
n-Propyl	1: 7,500	93.8	1: 7,000	77.8	1:12,000	80.0
n-Butyl	1: 18,000	257.0	1: 20,000	250.0	1:25,000	167.0
n-Amyl	1: 40,000	500.0	1: 50,000	556.0	1:40,000	367.0
Sec. Amyl	1: 25,000	312.0	1: 25,000	312.0	1:20,000	142.0
n-Hexyl	1:100,000	1,250.0	1:120,000	1,333.0	1:60,000	400.0
Cyclohexyl	1: 35,000	438.0	1: 32,500	361.0	1:40,000	250.0
n-Heptyl	1:120,000	1,500.0	1:200,000	2,222.0	1:80,000	533.0
n-Octyl	1:140,000	1,750.0	<1: 25,000	>312.0	<1:25,000	>167.0
Phenol (control)	1:70-80	1.0	1:80-90	1.0	1:140-160	1.0

While the cocci show greater resistance to the unsubstituted parachlorophenol and its lower homologues than the typhoid colon organisms (as evidenced by the higher concentrations required to kill the former), the conditions are reversed with the further increase of the molecular weight; now the coccidal efficiency increases until in the case of the n-heptyl derivative, the three cocci are killed in extremely low concentrations which, like the corresponding phenol coefficients, belong to an order of magnitude much higher than that of the preceding table. While the n-octyl p-chlorophenol is more effective against *Staph. aureus* than the n-heptyl derivative, its potency is less than that of the latter with respect to the other two cocci of Table II. It is to be noted especially that the greatest coccidal potency is shown by those derivatives which exert little or no effect upon the four organisms of the colon typhoid group previously referred to (Table I).

TABLE III

THE BACTERICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL UPON ACID-FAST BACTERIA. MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	MYCOBACTERIUM TUBERCULOSIS (HOMINIS)		MYCOBACTERIUM TUBERCULOSIS (AVIUM)		MYCOBACTERIUM LEPRAE (HOMINIS)		MYCOBACTERIUM LEPRAE (MURIUM)		MYCOBACTERIUM SMEGMATIS	
	I	II	I	II	I	II	I	II	I	II
p-Chlorophenol	1: 350	3.9	1: 400	4.0	1: 400	4.4	1: 350	3.9	1: 350	3.9
Alkyl Radical										
Methyl	1: 1,000	11.1	1: 1,000	11.1	1: 1,000	11.1	1: 900	10.0	1: 1,200	13.3
Ethyl	1: 2,500	27.8	1: 3,000	30.0	1: 2,000	22.2	1: 2,000	20.0	1: 2,500	25.0
n-Propyl	1: 6,000	66.7	1: 7,000	70.0	1: 6,000	66.7	1: 6,000	66.7	1: 8,000	88.9
n-Butyl	1:16,000	178.0	1:14,000	156.0	1: 16,000	178.0	1:20,000	200.0	1: 14,000	156.0
n-Amyl	1:35,000	389.0	1:30,000	333.0	1: 35,000	350.0	1:40,000	400.0	1: 40,000	400.0
Sec. Amyl	1:20,000	222.0	1:25,000	277.0	1: 25,000	278.0	1:30 000	333.0	1: 25,000	278.0
n-Hexyl	1:30,000	333.0	1:35,000	380 0	1: 60,000	600.0	1:70,000	700.0	1:100,000	1,111.0
Cyclohexyl	1:25,000	278.0	1:25,000	278 0	1: 25,000	278.0	1:30,000	333.0	1: 25,000	278.0
n-Heptyl	<1:40,000	>400.0	<1:60,000	>600.0	<1:100,000	>1,000.0	<1:40,000	>400.0	1:100,000	1.250.0
Phenol (control)	1:90-100	1.0	1:90-100	1 0	1:90-100	1.0	1:90-100	1.0	1:90-100	1.0

The susceptibility of the acid-fast bacteria (Table III) to the action of the monoalkyl homologues of parachlorophenol resembles that of the cocci in Table II both in a qualitative and a quantitative sense. The extraordinarily great bactericidal efficacy of the higher homologues against the organisms of the acid-fast group is particularly noteworthy in view of their reputed resistance to the action of phenolic disinfectants.

The "quasi-specific" feature of the germicidal action of the higher homologous orthoalkyl parachlorophenols has a parallel in the differentiation of

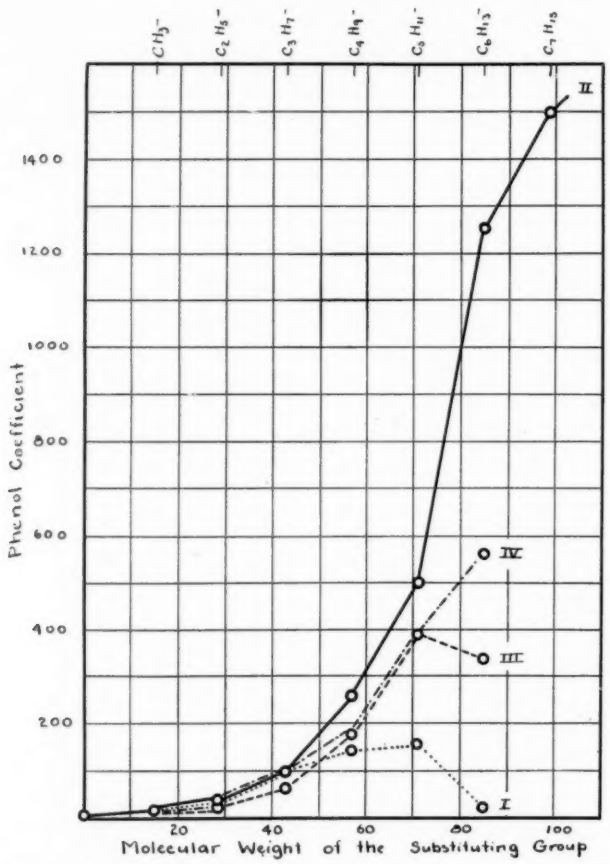


Chart 1.—The germicidal action of orthoalkyl derivatives of parachlorophenol. Test organisms: I, *Eberthella typhi;* II, *Staphylococcus aureus;* III, *Mycobacterium tuberculosis* (hom.); IV, *Monilia albicans.*

bacteria by means of the Gram stain and also in the action of certain basic dyes upon bacteria.[5, 6] More particularly, it appears that a gram-positive character and a susceptibility to the homologues with a higher molecular weight, run parallel in most but not in all cases. Thus *Micrococcus catarrhalis* forms an exception, in that in spite of its negative Gram reaction, it behaves much like the gram-positive organisms in this respect. However, it shows a similar behavior toward certain dyes. By way of advance information, it may be stated that *Vibrio cholerae* reacts essentially in the same manner to the higher homologues of this and other series of phenol derivatives, in spite

of its gram-negative character. Thus, while there are several examples of gram-negative microorganisms behaving like those of a positive Gram character in regard to their selective susceptibility to the "quasi-specific" action of the higher homologues of "phenolic" compounds, we do not know, to date, of any case of a gram-positive microorganism behaving under these conditions like the gram-negative bacteria of the typhoid colon group.

In spite of the entirely different morphologic character of the pathogenic fungi dealt with in Table IV, one is confronted again by the regularity in the functional reaction between the chemical constitution and fungicidal action, and by the somewhat astonishing qualitative and quantitative resemblance of this relation with that observed in the case of the cocci in Table II and of the acid-fast bacteria in Table III. Considered by itself, Table IV reveals the presence of several extremely effective fungicides in this series of compounds.

TABLE IV

THE FUNGICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL. MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	ACHORION SCHÖNLEINII		TRICHOPHYTON ROSACEUM		MONILIA ALBICANS	
	I	II	I	II	I	II
p-Chlorophenol	1: 300	3.3	1: 500	4.2	1: 400	4.0
Alkyl Group						
Methyl	1: 500	7.1	1: 1,400	11.7	1: 1,000	11.1
Ethyl	1: 2,250	25.0	1: 2,750	27.5	1: 3,250	32.5
n-Propyl	1: 5,000	714.0	1:10,000	83.3	1:10,000	100.0
n-Butyl	1:14,000	156.0	1:16,000	160.0	1:16,000	178.0
n-Amyl	1:25,000	278.0	1:40,000	400.0	1:35,000	389.0
Sec. Amyl	1:16,000	229.0	1:30,000	250.0	1:20,000	182.0
n-Hexyl	1:25.000	357.0	1:50,000	500.0	1:50,000	556.0
Cyclohexyl	1:20,000	222.0	1:50,000	417.0	1:30,000	300.0
n-Heptyl	1:14,000	175.0	1:60,000	667.0	<1:40,000	>363.0
Phenol (control)	1:70-90	1.0	1:90-110	1.0	1:90-110	1.0

Another illustration of the existence of a selective, "quasi-specific" effect is offered by the graphic presentation in Chart 1 in which a set of data obtained with one representative organism selected from each of the Tables I to IV has been used, viz., *Eberthella typhi, Staph. aureus, Mycobact. tuberculosis (hom.)*, and *Monilia albicans*. This chart also illuminates the difference in the order of magnitude of the bactericidal potencies with respect to the several groups of microorganisms under discussion.

POLYALKYL DERIVATIVES

As Table V indicates, this group of compounds (listed in the order of increasing molecular weight) also comprises some highly effective microbicides. The relation between the chemical constitution and germicidal action, while being distinctly in evidence, is not of a sufficiently direct character to permit graphic presentation. Here, too, as in the monoalkyl series, the weight of the substituting groups determines the selective, "quasi-specific" action.

TABLE V

THE GERMICIDAL ACTION OF POLYALKYL DERIVATIVES OF PARACHLOROPHENOL.

MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

DERIVATIVES OF 4-CHLORO-PHENOL	EBERTHELLA TYPHI		STAPHYLOCOCCUS AUREUS		STREPTOCOCCUS (HEMOL. STRAIN)		MYCOBACTERIUM SMEGMATIS		TRICHOPHYTON ROSACEUM	
	I	II	I	II	I	II	I	II	I	II
3-Methyl-	1: 1,500	10.7	1: 900	11.3	1: 900	11.3	1: 1,000	11.1	1: 1,200	11.0
3, 5-Dimethyl-	1: 4,500	30.0	1: 1,800	25.7	1: 2,750	27.5	1: 2,250	28.1	1: 2,500	25.0
6-Ethyl-3-methyl-	1: 9,000	64.3	1: 4,000	50.0	1: 5,000	55.6	1: 5,000	55.6	1: 6,000	60.0
6-n-Propyl-3-methyl-	1:20,000	133.0	1:16,000	200.0	1:16,000	178.0	1:14,000	156.0	1:15,000	150.0
6-iso-Propyl-3-methyl-	1:16,000	107.0	1:12,000	150.0	1:11,000	138.0	1:11,000	138.0	1:14,000	140.0
2-Ethyl-3, 5-dimethyl-	1: 7,500	46.4	1: 8,500	106.0	1: 8,500	94.4	1:11,000	122.0	1:13,000	130.0
6-sec. Butyl-3-methyl-	1: 7,500	50.0	1:40,000	500.0	1:32,000	361.0	1:35,000	389.0	1:40,000	364.0
2-iso-Propyl-3, 5-dimethyl-	1:13,000	81.3	1:25,000	313.0	1:25,000	313.0	1:32,500	325.0	1:27,500	275.0
6-Diethylmethyl-3-methyl-	1: 3,500	23.3	1:50,000	625.0	1:55,000	611.0	1:70,000	777.0	1:50,000	455.0
6-iso-Propyl-2-ethyl-3-methyl-	1: 8,500	56.7	1:16,000	200.0	1:14,000	175.0	1:18,000	200.0	1:16,000	145.0
2-sec. Butyl-3, 5-dimethyl-	1: 4,000	28.6	1:45,000	563.0	1:50,000	556.0	1:50,000	556.0	1:60,000	545.0
2-sec. Amyl-3, 5-dimethyl-	(1: 2,500)	(15.6)	1:60,000	750.0	1:80,000	889.0	1:70,000	700.0	<1:40,000	>400.0
2-Diethylmethyl-3, 5-dimethyl-	>1: 2,000	<13.0	1:80,000	1,143.0	1:80,000	1,000.0	1:60,000	667.0	1:70,000	700.0
6-sec. Octyl-3-methyl-	(1: 3,000)	(21.4)	<1: 8,000	>89.0	1:11,000	122.0	<1: 7,000	>70.0	>1: 8,000	<72.0
Phenol (control)	1:140-150	1.0	1:70-80	1.0	1:80-90	1.0	1:90-100	1.0	1:90-110	1.0

As to the effect upon the germicidal action, of the distribution of the added weight over several substituting radicals, a comparison, e.g., of the substituted parachlorophenol derivatives with a total of four substituting carbon atoms will furnish the necessary illustration. It shows that substitution of parachlorophenol by one alkyl group generally leads to a more effective compound than substitution by two or three alkyl groups with the same total number of carbon atoms; similarly, dialkyl substituted derivatives are considerably more germicidal than trialkyl substituted ones.

It is found in this group, too, that isoalkyl substituted compounds are less effective than the corresponding normal alkyl derivatives. This follows from the comparison of 6-n-propyl-3-methyl-4-chlorophenol and 6-isopropyl-3-methyl-4-chlorophenol (chlorothymol); thus the n-propyl derivative is distinctly more potent than chlorothymol.

Polyalkyl compounds of equal molecular weight, containing isoalkyl groups may show considerable variation in their germicidal action, depending upon their structure.

Whereas in the case of the normal monoalkyl derivatives the maximum effect upon *Eberthella typhi* was shown by a compound with five carbon atoms in the side chain, in the case of the polyalkyl derivatives this maximum is reached by a compound with a total of four substituting carbon atoms. As to the other test organisms in Table V the derivatives with a total of seven carbon atoms generally show the greatest microbicidal efficacy.

AROMATIC DERIVATIVES

The aromatic derivatives of parachlorophenol prepared and studied to date comprise the benzyl, and phenylethyl derivatives of parachlorophenol and its methyl homologues listed in Table VI. Because of the greater weight of the aromatic radicals, such substitution might be expected to produce compounds which in their antibacterial behavior would resemble the higher alkyl homologues, i.e., manifest a selective or "quasi-specific" action upon gram-positive and those other organisms which show an analogous behavior in this regard. In keeping with the general idea that particularly the molecular weight determines the point at which the "quasi-specific" germicidal effect becomes apparent, it is found that actually the compounds of this group show a selective action in the above sense, as evidenced by the numerical values of their germicidal potency.

There are among the aromatic derivatives some highly potent germicides. As to the effect of the chemical constitution upon germicidal action, it is noteworthy that the step from the benzyl derivative to the next one with a higher molecular weight is accompanied by different results depending upon the place in the molecular structure to which the additional weight is attached. Thus, 5-methyl-2-benzyl-4-chlorophenol is much less effective than 2-benzyl-4-chlorophenol against the four test organisms of the typhoid-colon group, while 2-phenylethyl-4-chlorophenol is very much more effective than the former. With reference to the other organisms, the difference in the germicidal potencies of the methyl-benzyl and the phenylethyl derivatives is less pronounced.

TABLE VI

THE GERMICIDAL ACTION OF AROMATIC DERIVATIVES OF PARACHLOROPHENOL.
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

DERIVATIVES OF 4-CHLOROPHENOL	EBERTHELLA TYPHI		EBERTHELLA PARADYSENTERIAE		SALMONELLA SCHOTTMÜLLERI (B. PARATYPHOSUS B)		ESCHERICHIA COLI	
	I	II	I	II	I	II	I	II
2-Benzyl-	1:10,000	71.4	1:12,000	92.3	1:10,000	71.4	1:10,000	71.4
5-Methyl-2-benzyl-	1: 2,750	18.3	1: 4,500	34.6	1: 4,000	28.6	(1: 2,500)	(19.2)
2-Phenylethyl-	1:16,000	100.0	1:12,000	80.0	1:10,000	71.4	1: 5,000	35.7
3, 5-Dimethyl-2-benzyl-	(1: 4,500)	(32.1)	1: 5,000	33.3	(1: 3,000)	(21.4)	(1: 2,500)	(17.9)
Phenol (control)	1:140-150	1.0	1:140-160	1.0	1:140-150	1.0	1:140-150	1.0

DERIVATIVES OF 4-CHLOROPHENOL	STAPHYLOCOCCUS AUREUS		STREPTOCOCCUS (HEMOL. STRAIN)		MYCOBACTERIUM TUBERCULOSIS (HOM.)		MYCOBACTERIUM LEPRAE (MUR.)		MYCOBACTERIUM SMEGMATIS		TRICHOPHYTON ROSACEUM	
	I	II	I	II	I	II	I	II	I	II	I	II
2-Benzyl-	1:16,000	200	1:18,000	225	1:16,000	178	1:20,000	222	1:20,000	222	1:30,000	273
5-Methyl-2-benzyl-	1:30,000	375	1:35,000	389	indefinite		indefinite		1:25,000	278	1:40,000	400
2-Phenylethyl-	1:30,000	375	1:40,000	500	1:30,000	333	1:30,000	333	1:37,500	375	1:37,500	375
3, 5-Dimethyl-2-benzyl-	1:60,000	750	1:70,000	778	indefinite		indefinite		1:30,000	333	1:70,000	636
3-Methyl-6-phenylethyl-	1:30,000	375	1:25,000	250	1:16,000	178	1:20,000	222	1:20,000	222	1:40,000	364
Phenol (control)	1:70-80	1	1:80-90	1	1:90-100	1	1:90-100	1	1:90-100	1	1:100-110	1

A further increase in the molecular weight lowers the germicidal action with reference to the bacteria of the typhoid-colon group as shown by the figures obtained with 3, 5-dimethyl-2-benzyl-4-chlorophenol. However, the germicidal potency is increased considerably with regard to the other four organisms. Curiously, 3-methyl-6-phenylethyl-4-chlorophenol, which is isomeric with 3, 5-dimethyl-2-benzyl-4-chlorophenol, is not only less effective than the latter, but also, in most instances, weaker than the 2-phenyl-ethyl derivative.

BACTERIOSTATIC ACTION

In addition to being potent germicides, certain substituted chlorophenol derivatives manifest considerable inhibitory capacity as well. An investigation of the bacteriostatic effect upon *Staphylococcus aureus* and upon three mycobacteria, viz., smegmatis, tuberculosis (hom.), and leprae (hom.) was carried out with 2-n-amyl-4-chlorophenol and 2-n-heptyl-4-chlorophenol. This experiment was conducted in such a manner as to permit the determination not only of the minimum concentrations required to prevent the growth of the microorganisms, but also that of the status of their viability during the course of their exposure to the inhibitory concentrations. This was accomplished by making transfers from the "inhibitory" media into fresh media after different periods of time.

The results obtained with the two compounds and the four organisms mentioned above are given in Table VII. It is to be noted among other things that whereas in the case of the 2-n-amyl-4-chlorophenol the minimum germicidal concentration is practically four times the minimum inhibitory one, in the case of 2-n-heptyl-4-chlorophenol it is only twice the latter (in the two cases where such comparison can be made).

The effect of P_H of the medium upon the inhibition by 2-n-amyl-4-chlorophenol and 2-cyclohexyl-4-chlorophenol of the growth of *Staph. aureus* and of *Mycobact. tuberculosis (hom.)* is illustrated in Table VIII. It is found that variations of P_H in both directions (i.e., to the acid and the alkaline sides) have little effect upon the bacteriostatic capacity of these compounds, a result of distinct practical significance.

TOXICITY

It is natural that the discovery of compounds showing such intensive antibacterial properties, should suggest the extension of this study beyond the stage of "in vitro" experiments, and encourage the inquiry into their availability for chemotherapeutic purposes. Some phases of this work are being planned at this time, others are under way.

One important condition of the potential usefulness of a drug for such purposes is a low organotropic against a high parasitotropic action. A preliminary series of experiments aiming at the determination of toxicity of the series of alkyl parachlorophenol derivatives (upon subcutaneous injection in mice*) shows that this toxicity decreases with increasing molecular weight

*For the purpose of this test the compounds in question were emulsified in a 0.5 per cent tragacanth solution, the proportion of the total quantity of fluid injected to the weight of the animal being approximately the same in all cases.

TABLE VII

BACTERIOSTATIC ACTION

A—2-N-AMYL-4-CHLOROPHENOL
B—2-N-HEPTYL-4-CHLOROPHENOL

MICROORGANISM	COMPOUND	TIME OF OBSERVATION IN HOURS	MINIMUM INHIBITORY CONCENTRATIONS	NO GROWTH IN SUBCULTURES FROM THE FOLLOWING CONCENTRATIONS AFTER							
				15 MIN.	1 HOUR	2 HOURS	4 HOURS	6 HOURS	24 HOURS	48 HOURS	72 HOURS
Staphylococcus aureus	A	96	1:150,000	1:40,000	1:50,000	1:60,000	1:60,000	1:80,000	1:120,000	1:150,000	1:150,000
	B		1:200,000	1:120,000	1:150,000	1:150,000	1:150,000	1:150,000	1:200,000	1:200,000	1:200,000
Mycobacterium smegmatis	A	120	1:200,000	1:50,000	1:80,000	1:80,000	1:120,000	1:120,000	1:150,000	1:150,000	1:200,000
	B		1:200,000	1:120,000	1:120,000	1:150,000	1:150,000	1:150,000	1:200,000	1:200,000	1:200,000
Mycobacterium tuberculosis (hominis)	A	168	1:150,000	1:30,000	1:40,000	1:40,000	1:50,000	1:60,000	1:150,000	1:150,000	1:150,000
	B		1:200,000	1:80,000	1:80,000	1:100,000	1:150,000	1:150,000	1:200,000	1:200,000	1:200,000
Mycobacterium leprae (hominis)	A	168	1:150,000	1:40,000	1:50,000	1:50,000	1:50,000	1:60,000	1:100,000	1:150,000	1:150,000
	B		1:200,000	1:80,000	1:100,000	1:100,000	1:100,000	1:100,000	1:120,000	1:150,000	1:200,000

(Table IX); in other words increasing antibacterial potency (with reference to most of the test organisms) is accompanied by decreasing toxicity. Thus in the case of several of the higher homologues, the enormous dose of 20 mg. per gm. of body weight (corresponding to about 1.5 kg. for an adult human being of 150 pounds) is tolerated. Even though it is inadmissible to draw direct conclusions as to the probable pharmacologic effect upon one species from the observed effect upon another, the preliminary toxicity experiments, too, indicate that the extension of this inquiry into the therapeutic field is not without some promise of success.

EXPERIMENTAL PART

Determination of Bactericidal Action.—All tests were carried out at 37° C. In all cases 0.5 c.c. of culture was added to 5 c.c. of the various dilutions of the antiseptic substances.

As to the test organisms of the typhoid colon group, cultures of *Eberthella typhi* were procured from the Bacteriological Laboratory of the Food and Drug Administration of the U. S. Department of Agriculture, those of *Eberthella paradysenteriae, Salmonella schottmülleri,* and *Escherichia coli* from the American Type Culture Collection. The method of culturing and preparing these organisms for the tests followed closely that described by Ruehle and Brewer[7] in the case of *Eberthella typhi,* and the testing technic used was the same as given in detail by these authors, i.e., transfers from the medication tubes into fresh media were made after exposures of five, ten, and fifteen minutes, respectively, and the readings were taken after forty-eight hours' incubation. For the sake of space economy the results of the five- and fifteen-minute exposures are left out in Table I (and incidentally also in Tables II to VI), and those of the ten-minute exposures only are given. The "phenol coefficients" were calculated in all cases from the results of the ten-minute exposures to the compounds under discussion on one hand, and to phenol (carbolic acid) on the other. A phenol control test always accompanied the tests with these compounds, carried out on any given day.

As to the three species of pathogenic cocci, the stock culture of *Staphylococcus aureus* was obtained from the Bacteriological Laboratory of the Food and Drug Administration, those of streptococcus and of *Micrococcus catarrhalis* from the Lederle Laboratories. *Staphylococcus aureus* was grown and applied in the test according to the directions given in Ruehle's and Brewer's publication, previously referred to. The same methods were applied in the case of *Micrococcus catarrhalis* yielding results of very satisfactory regularity.

The hemolytic strain of streptococcus used was one of considerable resistance, approaching that of *Staphylococcus aureus,* as evidenced by the results of the phenol-control tests. According to information received from Mr. E. F. Voight, bacteriologist of the Lederle Laboratories, whose assistance is gratefully acknowledged herewith, this strain was isolated originally from a case of scarlet fever and was selected as a scarlet fever type by Dr. A. R. Dochez at Johns Hopkins University. The stock culture for the test was grown on beef infusion peptone broth adjusted to P_H 7.2-7.4 and was used when twenty-

four hours old. After exposure to the various dilutions of the antiseptics, transfers were made into beef extract peptone broth containing 0.5 per cent of glucose and adjusted to P_H 7.2-7.4.

In the group of the acid-fast bacteria the strains of human and avian tuberculosis and those of human and rat leprosy were procured (with several others, not yet utilized in this investigation) from the U. S. Marine Hospital in Carville, Louisiana, through the courtesy of Dr. O. E. Denney to whom the authors wish to express their gratitude at this opportunity. The four original culture tubes received, were described as follows:

M. tuberculosis hominis Koch
M. tuberculosis avium Arlong (Phipps)
M. leprae human Clegg 1, Duval No. 105
M. leprae murium, Nat. Inst. Health

These four cultures were chosen because of the rapid growth which could be detected easily twenty-four hours after seeding. The medium for both stock cultures and subcultures was beef extract peptone broth containing 5 per cent of glycerin and adjusted to P_H 7.4. The stock cultures were grown for from five to seven days in strong bottles containing glass beads. Shortly before the test the bottles were stoppered with sterile rubber stoppers and shaken until a homogeneous suspension was obtained. This suspension was then poured through a sterile 200 mesh monel metal sieve in order to eliminate the larger particles. Final readings were made after incubation for seven days.

The original strain of *Mycobacterium smegmatis* was obtained from the American Type Culture Collection. It was grown and applied in the same manner as *Staphylococcus aureus* except that the stock emulsion for the test was forty-eight hours old and that final readings of the subcultures were made after one week's incubation.

As to the group of pathogenic fungi, the cultures of *Achorion schönleinii* and of *Monilia albicans* were procured from the American Type Culture Collection. The authors are very grateful to Dr. E. D. Osborne of Buffalo, N. Y., for two cultures of trichophyton, one of which was used in this work.

Achorion schönleinii and *Trichophyton rosaceum* were treated in the same manner. Both were grown on Sabouraud's agar which was also used for subcultures. The stock suspension for the test was prepared in the following manner: The growth of from ten to fifteen days was removed from the slant by means of a hooked platinum wire and placed in a sterile bottle with a layer of glass beads at the bottom. Four cubic centimeters of sterile normal saline was added, the bottle stoppered with a sterile rubber stopper and shaken vigorously, until the mass was broken up completely. Then another 6 c.c. of saline was added and the bottle shaken again. The suspension thus obtained was poured into a glass tube and the latter centrifuged for one minute at the lowest speed, in order to allow the large unbroken particles to settle to the bottom. The supernatant suspension was removed with a sterile pipette and diluted with double the amount of saline. As a rule the yield

from each culture tube was about 15 c.c. of suspension ready for use. The subcultures were held at 28° C. for ten days before the final readings were made.

Monilia albicans was also grown and subcultured on Sabouraud's agar. The suspension in normal saline was obtained by washing off a three- to five-day-old growth from the surface of the slant. Each tube gave 10 c.c. of the suspension for the test. Readings were made after incubation at 28° C. for five days.

DETERMINATION OF BACTERIOSTATIC ACTION

Regular bacteriostatic tests were carried out in the customary fashion by inoculation of media containing different concentrations of the antiseptic substances, and observation of the incubated tubes.

Where it was desired to determine the status of viability of the microorganisms in the presence of bacteriostatic concentrations, in relation to the time of exposure, the following procedure was used: To 9 c.c. of broth (of the same composition as used in the bactericidal tests) 1 c.c. of the antiseptic solution was added, containing ten times the concentration which appears in Tables VIII and IX. A spiral loop (of five turns of 2.18 mm. inside diameter, wire gage 23B and S., and holding approximately 0.02 c.c.) was used to plant the culture in this mixture previously warmed to 37° C. The tubes were kept

TABLE VIII

THE EFFECT OF P_H UPON THE BACTERIOSTATIC POTENCY. MINIMUM BACTERIOSTATIC CONCENTRATIONS IN FORTY-EIGHT HOURS AT 37° C.

	STAPHYLOCOCCUS AUREUS			MYCOBACTERIUM TUBERCULOSIS (HUMAN)		
	P_H 6.0	P_H 6.8	P_H 7.6	P_H 6.6	P_H 7.4	P_H 8.2
2-n-Amyl-4-chlorophenol	1:200,000	1:200,000	1:150,000	1:150,000	1:150,000	1:150,000
2-Cyclohexyl-4-chlorophenol	1:70,000	1:70,000	1:100,000	1:150,000	1:150,000	1:150,000

TABLE IX

TOXICITY OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL. SUBCUTANEOUS INJECTION IN MICE

MINIMUM LETHAL DOSES IN MILLIGRAMS PER GRAM OF BODY WEIGHT

DERIVATIVES OF 4-CHLOROPHENOL	M. F. D. MG. PER GRAM
p-Chlorophenol	0.6
Methyl	2.0
Ethyl	4.0
n-Propyl	6.0
n-Butyl	15.0
n-Amyl	>20.0
sec. Amyl	15.0
n-Hexyl	>20.0
Cyclohexyl	>20.0
n-Heptyl	>20.0

in the incubator for the stated periods of time and transfers were made into fresh media after fifteen minutes, one, two, four, six, twenty-four, forty-eight, and seventy-two hours with a loop of the same size. Final readings of the subcultures were made after incubation for forty-eight hours in the case of *Staphylococcus aureus*, and after one week in that of the acid-fast bacteria.

It is of possible practical interest that according to the outcome of several tests, both stock solutions and emulsions of the compounds under discussion, and also the final dilutions in the medication tubes may be sterilized in the autoclave without suffering any impairment of their bactericidal potency.

SUMMARY

The bactericidal and fungicidal action of the homologous series of parachlorophenol derivatives has been investigated with the aid of the following microorganisms: *Eberthella typhi, Escherichia coli, Eberthella paradysenteriae* (Flexner), *Salmonella schottmülleri, Staphylococcus aureus,* streptococcus (hemol. strain), *Micrococcus catarrhalis, Mycobacterium tuberculosis (hom.)* and *(avium), Mycobacterium leprae (hom.)* and *(mur.), M. smegmatis, Monilia albicans, Trichophyton rosaceum* and *Achorion schönleinii.*

In confirmation of previous preliminary findings (stated in the initial paragraphs of this paper), the presence in this series of some derivatives of an extraordinarily high bactericidal and fungicidal potency has been established. The relationship between the chemical constitution and antibacterial action has been discussed in some detail. A "quasi-specific" effect of certain higher alkyl homologues upon the test organisms, exclusive of the bacteria of the typhoid-colon group, appears to divide the microorganisms in two groups, the division agreeing partly, but not entirely with that obtained by means of the Gram stain, and evidently also with that based upon their susceptibility to the inhibitory action of certain basic dyes. Nevertheless, the class of compounds under consideration comprises some bactericides, highly effective against the organisms of the typhoid colon group as well.

In addition to the monoalkyl derivatives, several polyalkyl and aromatic compounds were studied and the regularities in their antibacterial behavior were pointed out.

The bacteriostatic potency of some of the derivatives was also investigated and found to exist to a remarkable degree not only with respect to staphylococcus, but also with respect to several acid-fast strains. The comparative independence of the bacteriostatic capacity from the P_H within rather wide limits was emphasized as being of possible practical significance.

This great antibacterial efficacy of certain derivatives of the homologous parachlorophenol series was found to be accompanied by very low toxicity to mice. Specifically, the increase in weight of the substituting alkyl radical which causes, within certain limits, an increase in the germicidal potency, produces also a sharp decline in the toxicity to animals. This combination of high antibacterial capacity and low toxicity to animals, as encountered in several instances, suggests the extension of the present inquiry into the field of chemotherapy.

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NORMAL ERYTHROCYTE, HEMOGLOBIN AND PACKED CELL VOLUME STANDARDS IN YOUNG MEN*

A Study of 100 Subjects

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THE unsatisfactory character of the data on which the long-accepted normal erythrocyte and hemoglobin values are based has been pointed out frequently in recent years. The increasing use of the blood indices or of the average volume and hemoglobin content of the individual erythrocyte in differentiating anemias has emphasized the need of dependable normal values. In an effort to provide adequate data for reliable standards, a number of extensive studies have been made on young men. Haden[9, 10] in 1922 published a study which included observations on 20 men between the ages of eighteen and thirty. This series was later increased to 35.[14] Osgood[24] in 1926 followed with data on the blood of 137 men between nineteen and thirty. Wintrobe and Miller[33] made similar observations on 100 men between the ages of twenty and thirty. Foster and Johnson[8] followed with a similar study of 115 normal men. Even with these data, general erythrocyte and hemoglobin standards are by no means established,[1, 5] and the need for additional study in other localities has often been pointed out.[12, 24, 36]

The present study is based upon the observations made on 100 men between the ages of twenty and thirty. The subjects were medical and college students, all of whom had been found in good health by thorough physical examination. Since previous investigation has shown that both muscular activity[17] and rest[32] produce significant fluctuations in the erythrocyte count, the samples of blood were drawn as soon as the subjects presented themselves at the laboratory.

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No other effort was made to control physical activity. The data are, therefore, believed to be comparable to previous studies in which the subjects have, also, apparently been chosen at random with respect to physical activity. Most of the observations were made during the month of September.

METHODS

A 6 c.c. quantity of blood was drawn from an arm vein, 5 c.c. being placed in a 15 c.c. graduated centrifuge tube containing 1 c.c. of 1.6 per cent sodium oxalate solution. The 1 c.c. of blood was placed in a second tube containing a small amount of dry oxalate and was later used for making counts and hemoglobin determinations. All tubes were tightly stoppered immediately, and samples were centrifuged or diluted before any known changes which would affect any of the observations could occur in the blood. Precautions were taken to prevent stasis in arm veins when withdrawing blood.

Cell volume was ascertained by centrifuging the tubes containing the 5 c.c. of blood in 1.6 per cent oxalate for one hour, which is considered adequate by Haden,[13] at a speed of 2,800 r.p.m. in a centrifuge with a working radius of 13.5 cm. The tubes were allowed to return to room temperature before readings were made. The percentage of cells was calculated from the readings of the graduated scale on the tube.

Erythrocyte counts were made after dilution in Trenner pipettes, in Levy-Hausser chambers having improved Neubauer ruling, all of which apparatus had been tested by the U. S. Bureau of Standards. The pipettes were agitated for three minutes in a mechanical shaker which was carefully checked to eliminate the possibility of any mechanical hemolysis. At least two dilutions with Hayem's solution were made from each sample and, if necessary, additional dilutions were made until counts from separate pipettes were obtained which agreed within 100,000 cells. Blood was thoroughly mixed by repeated inversion of tubes before samples were taken out, and diluted samples were allowed to settle completely in the chamber before counting. Trenner pipettes were checked with U. S. Standard Thoma pipettes and were found to give concordant readings.

Hemoglobin was estimated by the Newcomer method. Determinations were made after the acid hematin solution had stood for one hour. All readings were made by daylight. Each reading recorded is the average of six observations of the colorimeter. In order to verify the dependability of the

TABLE I

BLOOD SAMPLE NUMBER	NEWCOMER METHOD	OXYGEN CAPY. METHOD	VARIATION OF NEWCOMER
1	10.86	10.84	+0.02
2	11.48	10.89	+0.59
3	11.83	11.86	+0.03
4	13.35	13.34	+0.01
5	14.84	14.52	+0.32
6	15.23	15.24	−0.01
7	15.15	15.30	−0.15
8	16.12	15.79	+0.33
9	15.20	15.85	−0.65
10	25.78	25.81	−0.03

Newcomer disc, the apparatus was checked against the Van Slyke oxygen capacity method on 10 different samples of blood. The results were as shown in Table I.

Because of the close correspondence of this series of comparative determinations, no corrections were applied to the Newcomer readings. All cell counts and hemoglobin determinations were made by the same individual.

THE ERYTHROCYTE COUNT

Other investigators[24, 33] have reviewed the literature dealing with normal erythrocyte values and have shown how prevailing standards originated from a surprisingly small number of observations. In the United States, four important studies of normal men in the age range from eighteen to thirty, and using accurate methods for erythrocyte count, total hemoglobin and total cell volume have appeared recently.

Working in Kansas, Haden observed in a series of 20 men, aged eighteen to thirty, a mean erythrocyte count of 5.08. The lowest count recorded was

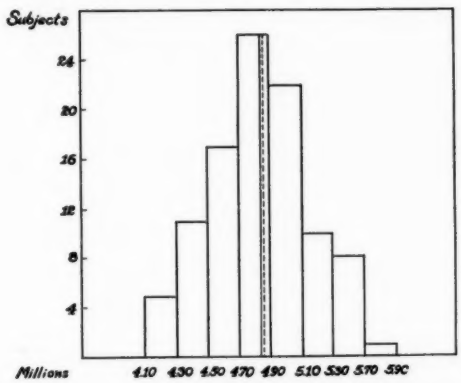

Fig. 1.—Frequency distribution of erythrocyte count in 100 young men. The median, 4.86, is indicated by a broken line and the mean, 4.84, by a solid line.

4.52 and the highest, 5.62. When the series was increased to 35 subjects, the mean count was 5.05 million. Osgood, in Oregon, found the mean erythrocyte count for the 137 men of his series to be 5.39 million, ranging from 4.4 to 6.4 million, with 90 per cent lying between 4.7 and 6.1 million. Wintrobe and Miller, working in Louisiana, observed a mean of 5.85 million in the 100 men examined by them, with 95 per cent between 5 and 6.90 million. The minimum and maximum values of this group were 4.68 and 7.53, respectively.

Emerson[7] in Baltimore reported the average count of 176 men between the ages of twenty and twenty-five as 5 million, with only 8 per cent below 5 million and 8.5 per cent above 6 million. Haden[15] has recently reported a series of counts made by medical students in Kansas City on their own blood, in which a mean of 4.997 was found, the average of 54 students in 1929 to 1930 being 4.85 million. Figures reported by various other investigators are given in Table II.

In the 100 men observed in the present study, the mean erythrocyte count was 4.84 million per cubic millimeter of blood, the counts ranging from 4.10 to 5.55 million. Of this number, 90 per cent lay between 4.31 and 5.35 million.

TOTAL HEMOGLOBIN AND COLOR INDEX

Haden, using the Van Slyke method, found in his study of 35 men a mean total hemoglobin of 15.63 gm. per 100 c.c. of blood. Osgood, employing the Osgood-Haskins method of hemoglobin determination, standardized by the Van Slyke, found a mean of 15.76 gm. in his series of 137 men. Ninety per cent of the values were between 14 and 18 gm. Wintrobe and Miller,[33, 34] using the Newcomer hemoglobinometer corrected by the Van Slyke method, obtained a mean hemoglobin figure of 17 for 100 male subjects, with 73 per cent of the determinations ranging between 15.5 and 18.5 gm. Foster and Johnson found a mean value of 15.63 gm. per 100 c.c. for 115 men.

Several large series of normal men have been investigated by workers in other localities. The mean values found by Harrop,[16] Orias,[21, 22] Parodi,[26] Sokhey,[30] Parjono et al.,[25] Tenconi,[31] Price-Jones,[28] Dill,[4] and more recently, by Jenkins and Don,[19] are shown in Table III.

The familiar work of Williamson is criticized[27] because a standard of recrystallized hemoglobin was used. Horneffer's[18] mean of 16.03 gm. per 100 c.c. for 40 men has been questioned[28] for the same cause.

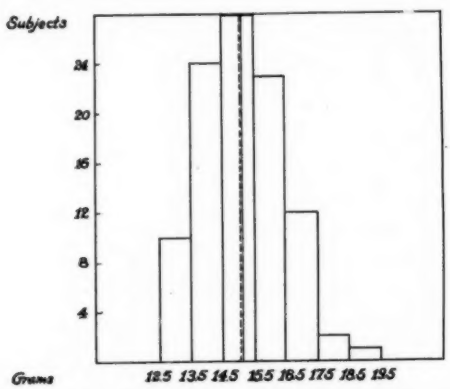

Fig. 2.—Frequency distribution of hemoglobin values in 100 young men. The median, 15.02, is indicated by a broken line and the mean, 15.12, by a solid line.

Various methods of determination have been reported. Sackett,[29] using the Wong iron method, found a mean value of 16.71 gm. per 100 c.c. in 15 normal men. Chia Yu Tien[31a] reports a series of observations on 321 healthy Chinese in which a mean of 97.4 per cent by the Sahli method was found. Komocki[20] records a mean of 86 per cent Sahli in 17 healthy men associated with an average red count of 5.97 million. Cabigting,[2] using the Bausch and Lomb hemoglobinometer, found a mean of 82.7 per cent for 22 normal male Filipinos.

The mean value found for the 100 subjects of the present study was 15.12 gm. per 100 c.c. of blood, 90 per cent of the figures lying between 13.25 and 17.03 gm. The extremes were 12.89 and 18.70 gm.

In the calculation of color index, it is of course necessary to employ an arbitrary standard for erythrocytes and hemoglobin which shall be regarded as 100 per cent. The use of 5 million is universally accepted as 100 per cent

of red cells. Osgood, on the basis of his own observations and the data of previous investigators, recommended the use of the hemoglobin coefficient, 14.7 gm., to represent 100 per cent. Wintrobe adopted 14.6 gm. as a more accurate figure to represent 100 per cent.

The inadaptability of a hemoglobin coefficient derived largely from one or two series to all localities is demonstrated by employing the figure used as 100 per cent by Wintrobe in four of the separate series being compared. The data of Osgood and of Wintrobe and Miller, when so treated, show a mean color index of 1. Haden's subjects show a mean color index of 1.07 and those of the present series, 1.08, with Wintrobe's 100 per cent figure. If, however, a hemoglobin coefficient is calculated from the combined data of Haden and of this study, the resulting figure, 15.59 gm., when used as 100 per cent hemoglobin, gives a mean color index of 1.006 for the subjects of the present series, 85 per cent of which are between 0.85 and 1.15.

TOTAL VOLUME OF PACKED CELLS AND VOLUME INDEX

Since the procedure followed by Osgood and by Wintrobe and Miller employed powdered oxalate as an anticoagulant, corrections were applied to

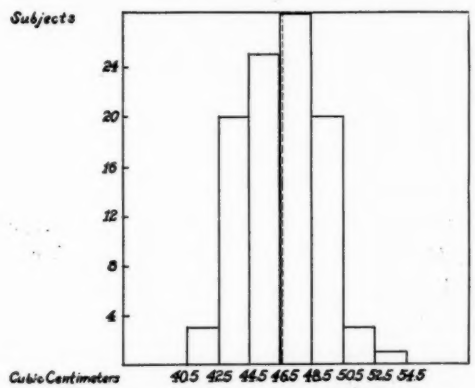

Fig. 3.—Frequency distribution of total volume of packed cells in 100 young men. The median, 46.8, is indicated by a broken line and the mean, 46.5, by a solid line.

their original figures. Osgood, using 20 mg. powdered oxalate to 10 c.c. of blood, obtained an average total volume of 44.84 c.c. of packed cells per 100 c.c. of blood in the 94 subjects who were so examined. This figure was increased by 3.5 per cent to correct for shrinkage of the cells, giving a revised figure of 46.4 c.c. packed cells per 100 c.c. of blood. Ninety per cent of the values ranged between 40 and 50 c.c.

The corrected mean of Wintrobe and Miller is 46.5 c.c. of packed cells per 100 c.c. of blood. This was obtained by adjusting for the 6.7 per cent shrinkage in volume with the 40 mg. of oxalate per 10 c.c. of blood used by them. Of the corrected values, 85 per cent ranged between 40 and 50 c.c.

The mean total cell volume found in the present series was 46.5 c.c. packed cells per 100 c.c. of blood, with 92 per cent of the total volume determinations between 43 and 50 c.c. This average agrees with Haden's first published data[10] in which 1.6 per cent oxalate solution was used as an anticoagulant.

Haden[13] has since demonstrated that 1.4 per cent oxalate is more nearly isotonic with human blood than 1.6 per cent although the difference is small. The mean value found by Foster and Johnson for 40 subjects using heparin as an anticoagulant is slightly greater, as might be expected. Peters and Van Slyke[27] mention the unpublished packed cell volume determinations of Eisenman as agreeing excellently with those of Haden and Osgood. Pearl and Miner[26a] report a mean of 45.59 c.c. in their study of 272 normal men.

In spite of the fact that the various average figures for total volume of packed cells agree so closely, the great divergence in red cell counts produces volume coefficients in each instance which are widely separated. Osgood's corrected volume coefficient is 43.04 c.c., while the high average cell count found in Wintrobe and Miller's subjects gives a volume coefficient of 39.94. Foster and Johnson calculated 44.4 as the volume coefficient of their 40 subjects. Osgood, as well as Wintrobe and Miller, employed 41 c.c. as 100 per cent in their calculations, giving volume indices of 1.00 and 0.97 respectively. The use of 41.0 c.c. as 100 per cent would give a volume index in Haden's group of 1.12 and in this series, of 1.18. Obviously, again, the standard chosen as 100 per cent must, judging from the above calculations, be a local one, at least for the present. If the data of Haden and of this series are combined, a volume coefficient of 47.4 c.c. is derived, which, when considered as 100 per cent total cell volume, gives a mean volume index in this series of 1.017 with 90 per cent between 0.89 and 1.15.

SATURATION INDEX

Employing the standards used by Wintrobe, a hemoglobin coefficient of 14.6 gm. and a volume coefficient of 41 c.c., the 100 subjects of Wintrobe and Miller average 1.02, the Oregon men, 0.98, and Haden's Kansas City group 0.96 in saturation index. The series under consideration would average 0.92. If, however, 15.59 gm. hemoglobin and 47.4 c.c. packed cells are taken as 100 per cent standards, the mean saturation index obtained is 0.99 in this series, with 90 per cent ranging between 0.85 and 1.12.

MEAN CORPUSCULAR VOLUME, HEMOGLOBIN AND HEMOGLOBIN CONCENTRATION

The method of calculating mean corpuscular volume, hemoglobin and hemoglobin concentration has been explained frequently elsewhere[10, 11, 35, 36] and is therefore omitted here. Mean corpuscular volume refers to the average volume of individual erythrocytes, stated in cubic micra. Mean corpuscular hemoglobin expresses the average amount of hemoglobin in micromicrograms (grams $\times 10^{-12}$) present in the erythrocytes. Mean corpuscular hemoglobin concentration expresses the amount of hemoglobin in proportion to the volume of the erythrocyte and is stated in percentage.

The mean corpuscular volume obtained in this study, 96.5 cubic micra, is higher than that found in any of the other series compared in Table IV. The normal range for the age group, sex, and locality of this study is found between 82 and 107 cubic micra, since 90 per cent of the observations occurred within those limits.

These figures tend to confirm the relationship suggested by the work of Emmons[6] on various mammalian as well as human bloods, that the volume of the individual erythrocyte appears to vary inversely with the total number found, a larger corpuscular volume tending to compensate for the smaller number of cells.

Haden's average for mean corpuscular volume in men is 92 cubic micra. Wintrobe found 79.8 cubic micra for an average, while the mean figure calculated from Osgood's data is 86.1 cubic micra. Foster and Johnson's figure is 85.9.

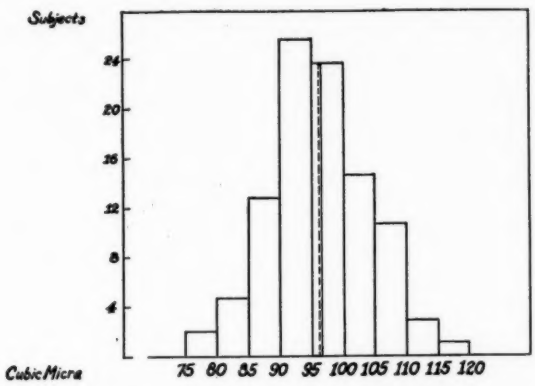

Fig. 4.—Frequency distribution of mean corpuscular volume in 100 young men. The median, 96.3, is indicated by a broken line and the mean, 96.5, by a solid line.

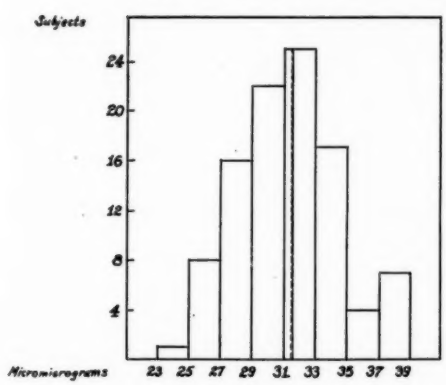

Fig. 5.

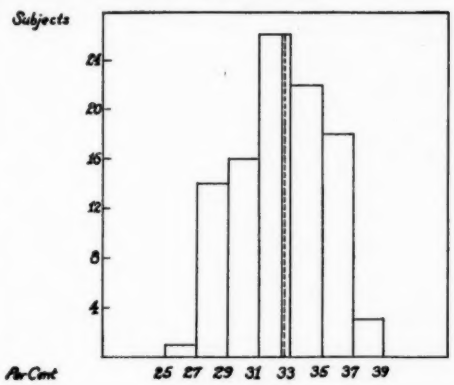

Fig. 6.

Fig. 5.—Frequency distribution of mean corpuscular hemoglobin in 100 young men. The median and the mean are identical, 31.4.

Fig. 6.—Frequency distribution of mean corpuscular hemoglobin concentration in 100 young men. The median, 32.6, is indicated by a broken line and the mean, 32.4, by a solid line.

Since the calculation of corpuscular volume, hemoglobin and hemoglobin concentration is simple and does not involve any arbitrary standard, these constants have been recommended frequently in preference to the volume, color, and saturation indices for clinical work. However, it is clear that even for the application of these terms to specific cases, well-established normals for the given conditions, age group, and sex should be known. For example, although Wintrobe[36] states that "values above 95 cubic micra or below 75 cubic micra are probably a manifestation of abnormality," more than half of the

normal subjects observed in the present investigation showed a corpuscular volume greater than 95 cubic micra.

The average figure for mean corpuscular hemoglobin found in the subjects of this study was 31.4 micromicrograms, with 92 per cent lying between 25 and 37 micromicrograms. This mean is almost identical with Haden's average of 31.0 micromicrograms for 35 men between eighteen and thirty, and agrees closely with the average figure found by Foster and Johnson, 30.03 micromicrograms. The same mean was observed both by Osgood and by Wintrobe and Miller, 29.2 micromicrograms.

The average found for corpuscular hemoglobin concentration was 32.4 per cent. Ninety-one per cent of the calculations lay between 27.5 and 36.5 per cent. Haden's average of 33.7 per cent and Osgood's, 33.9 per cent, are in close agreement with this figure. Wintrobe found an average of 36.6 per cent, while Foster and Johnson recorded a mean of 34.77. Horneffer calcu-

TABLE II

MEAN ERYTHROCYTE COUNT FOR NORMAL MEN IN VARIOUS GEOGRAPHIC LOCALITIES

INVESTIGATOR	DATE	NO. OF SUBJECTS	LOCALITY	MEAN COUNT	AGE RANGE
Chamberlain[3]	1911	687	Philippine Is.	5.20	----
Emerson	1921	176	Baltimore	5.00	20-25
Haden	1922 ff.	70	Kansas City	4.95	18-50
Haden	1924-1930	230	Kansas City	5.00*	----
Komocki	1924	17	Warsaw	5.97	20-40
Sackett	1925	14	Kansas City	5.09	----
Osgood	1926	137	Oregon	5.39	19-30
Horneffer	1928	40	Germany	4.97	19-29
Wintrobe and Miller	1929	100	New Orleans	5.85	20-30
Parodi	1930	50	Buenos Aires	5.50	18-30
Cabigting	1930	22	Philippine Is.	4.75	15 "up"
Foster and Johnson	1931	115	New Orleans	5.26	18-30
Tenconi	1931	50	Buenos Aires	5.30	----
Price-Jones	1931	100	London	5.43	----
Chia Yu Tien†	1931	320	Manchuria	5.12	19-64
This Study	1933	100	Kansas	4.84	20-30
Mean Erythrocyte Count for 2228 Men				5.189	

*These counts were made by medical students.
†Quotes Kitashima as having found 5.24 million and Sakai, 5.46 million as mean values in studies on normal Japanese.

TABLE IV

COMPARISON OF AVERAGE NORMAL ERYTHROCYTE, HEMOGLOBIN AND PACKED CELL VOLUME VALUES FOUND BY VARIOUS INVESTIGATORS

INVESTIGATOR	LOCALITY	NUMBER SUBJECTS	AGE RANGE	ERYTHROCYTES	TOTAL HB.	HB. COEFF.	TOTAL VOLUME	VOLUME COEFF.	CORP. VOL.	CORP. HB.	CORP. HB. CONC.
Haden	Kansas	35	18-30	5.05	15.63	15.48	46.2	46.0	92.0	31.0	33.7
Osgood	Oregon	137	19-30	5.39	15.76	14.66	46.4*	43.0	86.1	29.2	33.1
Wintrobe and Miller	New Orleans	100	20-30	5.85	17.0	14.53	46.5	39.9	79.8	29.2	36.6
Foster and Johnson	New Orleans	115	18-30	5.26†	15.63	14.85	46.7‡	44.4‡	88.8‡	30.0	34.8
This study	Kansas	100	20-30	4.84	15.12	15.71	46.5	48.3	96.5	31.4	32.4

*Mean of 94 Subjects.
†Mean of 100 Subjects.
‡Heparinized Blood. Mean of 40 Subjects.

TABLE III

MEAN HEMOGLOBIN VALUES FOR NORMAL MEN IN VARIOUS GEOGRAPHIC LOCALITIES

INVESTIGATOR	LOCALITY	DATE	NO. OF SUBJECTS	AGE RANGE	HEMOGLOBIN GM./100 C.C.	HEMOGLOBIN COEFF.	METHOD	REMARKS
Harrop	Baltimore	1919	15	----	15.05	----	Van Slyke	------
Haden	Kansas City	1922 ff.	70	18-50	15.34	15.50	Van Slyke	------
Osgood	Oregon	1926	137	19-30	15.76	14.66	Osgood-Haskins: Van Slyke	------
Wintrobe and Miller	New Orleans	1929	100	20-30	17.0	14.53	Newcomer: Van Slyke	------
Orias	Argentine	1930	1104	----	14.46	----	Newcomer: Van Slyke	Soldiers
Orias	Argentine	1930	65	----	15.06	----	Newcomer: Van Slyke	Officers
Orias	Argentine	1930	82	----	15.3	----	Newcomer: Van Slyke	Students
Parodi	Buenos Aires	1930	50	18-30	15.4	14.0	Newcomer	------
Sokhey	Bombay	1930	121	----	15.3	----	Van Slyke	------
Parjono, et al.	Java	1930	19	----	14.63	----	Van Slyke	Poorer natives
Parjono, et al.	Java	1930	16	----	16.12	----	Van Slyke	Native students and doctors
Foster and Johnson	New Orleans	1931	115	18-30	15.63	14.84	Van Slyke	------
Tenconi	Argentine	1931	50	----	14.8	13.9	Newcomer: Van Slyke	------
Price-Jones	London	1931	100	20-51	14.5	13.35	Van Slyke	------
Dill	Boston	1931	40	----	15.4	----	Van Slyke	------
Jenkins and Don	England	1933	118	----	15.85	----	Van Slyke	------
This Study	Kansas	1933	100	20-30	15.12	15.71	Newcomer: Van Slyke	------
Mean hemoglobin for 2302 men					14.987			
Mean hemoglobin for 1198 men (omitting 1104 Argentine soldiers)					15.472			
Mean hemoglobin coefficient for 722 men						14.618		

TABLE V

BLOOD FINDINGS IN 100 NORMAL MEN AGED 20 TO 30

NO.	AGE	ERYTHRO-CYTES	TOTAL HEMOGLOBIN	HEMOGLOBIN COEFF.	TOTAL CELL VOLUME	VOLUME COEFF.	CORPUSCULAR VOLUME	CORPUSCULAR HEMOGL.	CORPUSC. HEMOGL. CONCENTR.	COLOR INDEX*	VOLUME INDEX*	SATUR. INDEX*
1	22	4.31	13.44	15.59	48.8	56.6	113.2	31.2	27.5	1.00	1.19	0.83
2	21	5.33	13.44	12.61	46.6	43.7	87.4	25.2	28.8	0.81	0.92	0.88
3	21	5.29	15.64	14.78	44.0	41.6	83.1	29.6	35.5	0.94	0.88	1.08
4	28	4.83	15.90	16.46	48.9	50.6	101.2	32.5	32.5	1.05	1.05	0.99
5	22	4.93	15.64	15.86	44.6	45.2	90.4	31.7	35.1	1.02	0.96	1.06
6	20	4.72	17.03	18.04	46.1	48.8	97.7	36.1	36.9	1.15	1.03	1.12
7	23	4.33	15.90	18.36	43.6	50.3	100.7	36.7	36.5	1.17	1.06	1.11
8	27	4.72	14.03	14.86	44.6	47.2	94.5	29.7	31.5	0.95	0.99	0.96
9	20	4.36	16.74	19.20	43.5	49.9	99.8	38.4	38.4	1.22	1.06	1.16
10	20	5.09	14.90	14.62	45.3	44.5	89.0	29.3	32.9	0.94	0.94	1.00
11	21	4.66	16.17	17.35	46.8	50.2	100.4	34.7	34.6	1.11	1.06	1.05
12	20	4.47	15.39	17.22	43.4	48.6	97.1	34.4	35.5	1.11	1.03	1.08
13	23	4.10	15.90	19.39	44.8	54.6	109.3	38.8	35.5	1.24	1.16	1.07
14	20	4.77	16.45	17.24	47.9	50.2	100.4	34.5	34.3	1.11	1.06	1.05
15	20	4.40	14.90	16.93	41.1	46.7	93.4	33.9	36.3	1.09	0.99	1.10
16	20	5.05	15.90	15.74	50.0	49.5	99.0	31.4	31.8	1.01	1.04	0.97
17	22	4.29	14.90	17.37	47.4	55.3	110.5	34.7	31.4	1.12	1.16	0.96
18	20	5.38	15.90	14.78	48.8	45.3	90.7	29.6	32.6	0.95	0.96	0.99
19	21	4.96	17.03	17.17	45.7	46.1	92.1	34.3	27.3	1.10	0.97	1.14
20	20	4.91	16.17	16.47	48.8	49.7	99.4	32.9	33.1	1.06	1.05	1.01
21	20	4.53	17.66	19.49	47.0	51.9	103.8	39.0	37.6	1.24	1.09	1.14
22	21	5.45	15.14	13.89	43.1	39.5	79.1	27.8	35.1	0.89	0.83	1.07
23	21	4.56	17.34	19.01	50.0	54.8	109.6	38.0	34.7	1.22	1.15	1.06
24	20	4.62	17.34	18.77	48.7	52.7	105.4	37.5	35.6	1.21	1.12	1.08
25	20	4.50	15.14	16.82	45.0	50.0	100.0	33.6	33.6	1.08	1.06	1.02
26	20	5.00	15.90	15.90	44.4	44.4	88.8	31.8	35.8	1.02	0.94	1.09
27	20	5.31	13.44	12.66	44.2	41.6	83.2	25.3	30.4	0.81	0.88	0.92
28	22	4.75	15.64	16.46	45.7	48.1	96.2	32.9	34.2	1.05	1.01	1.04
29	21	4.83	13.63	14.11	43.1	44.6	89.2	28.2	28.2	0.90	0.94	0.96
30	20	4.54	15.64	17.23	43.3	47.7	95.4	34.4	36.1	1.10	1.00	1.10
31	25	4.28	15.90	18.58	44.6	52.1	104.2	37.1	35.7	1.18	1.09	1.08
32	20	4.82	14.90	15.46	42.2	43.8	87.6	30.9	35.3	1.00	0.93	1.08
33	22	4.75	15.64	16.46	46.8	49.3	98.5	32.9	33.4	1.05	1.04	1.01
34	20	4.56	15.90	17.44	48.0	52.6	105.3	34.9	33.1	1.12	1.11	1.01
35	22	4.91	16.74	17.05	50.0	50.9	101.8	34.1	33.5	1.09	1.07	1.02
36	20	4.50	15.14	16.82	43.9	48.8	97.6	33.6	34.5	1.08	1.03	1.04
37	21	4.42	13.63	15.42	47.1	53.3	106.6	30.8	28.9	0.99	1.13	0.88
38	20	4.60	14.90	16.20	46.5	50.5	101.1	32.4	32.0	1.04	1.06	0.98
39	20	4.52	13.07	14.46	47.9	53.0	106.0	28.9	27.3	0.92	1.11	0.83
40	21	4.72	17.34	18.37	46.8	49.6	99.2	36.7	37.1	1.18	1.05	1.12
41	23	5.06	15.14	14.96	46.1	45.6	91.1	29.9	32.8	0.96	0.96	1.00
42	21	4.72	14.22	15.05	43.7	46.3	92.6	30.1	32.5	0.97	0.98	0.99
43	25	5.13	13.82	13.46	42.7	41.6	83.2	26.9	32.4	0.86	0.87	0.99
44	21	5.08	14.44	14.21	47.4	46.7	93.3	28.4	30.5	0.92	0.99	0.93
45	26	4.88	14.22	14.53	46.8	48.0	95.9	29.1	30.4	0.93	1.01	0.92
46	22	5.55	16.45	14.82	47.0	42.3	84.7	29.6	35.0	0.95	0.89	1.07
47	21	4.56	13.44	14.74	41.1	45.1	90.1	29.5	32.7	0.95	0.96	0.99
48	20	5.01	14.67	14.64	48.4	48.3	96.6	29.3	30.3	0.94	1.02	0.92
49	22	5.10	13.63	13.36	45.9	45.0	90.0	26.7	29.9	0.85	0.95	0.90
50	28	5.33	13.82	12.95	49.4	46.3	92.7	25.9	28.0	0.83	0.97	0.86
51	23	4.49	14.03	15.59	47.4	52.8	105.6	31.2	29.6	1.00	1.11	0.90
52	24	4.88	15.14	15.51	48.9	50.1	100.2	31.0	31.0	0.99	1.05	0.94
53	20	4.89	16.74	17.12	50.0	51.1	102.2	34.2	33.5	1.08	1.07	1.02
54	26	4.88	15.90	16.29	43.9	45.0	90.0	32.6	36.2	1.04	0.95	1.09

*For the calculation of these indices, 15.59 gm. of hemoglobin and 47.4 c.c. of packed cells have been considered as 100 per cent.

TABLE V—CONT'D

NO.	AGE	ERYTHRO-CYTES	TOTAL HEMOGLOBIN	HEMOGLOBIN COEFF.	TOTAL CELL VOLUME	VOLUME COEFF.	CORPUSCULAR VOLUME	CORPUSCULAR HEMOGL.	CORPUSC. HEMOGL. CONCENTR.	COLOR INDEX*	VOLUME INDEX*	SATUR. INDEX*
55	23	4.99	17.03	17.06	49.6	49.7	99.4	34.1	34.3	1.09	1.05	1.04
56	25	4.54	15.14	16.68	46.9	51.7	103.3	33.3	32.3	1.07	1.09	0.98
57	21	4.27	13.63	15.96	46.8	54.8	109.6	31.9	29.1	1.02	1.16	0.88
58	21	4.88	13.25	13.58	43.5	44.6	89.1	27.2	30.5	0.87	0.94	0.92
59	21	4.97	15.64	15.73	45.9	46.2	92.4	31.7	34.1	1.01	0.98	1.03
60	23	4.80	15.39	16.03	44.7	46.6	93.1	32.1	34.4	1.03	0.98	1.05
61	20	4.74	13.63	14.38	43.5	45.9	91.8	28.8	31.3	0.92	0.97	0.95
62	20	4.67	13.44	14.39	45.6	48.8	97.6	28.8	29.5	0.92	1.03	0.90
63	20	5.03	14.03	13.95	47.5	47.2	94.4	27.9	29.5	0.89	0.99	0.90
64	20	4.89	14.44	14.77	48.9	50.0	100.0	29.5	29.5	0.95	1.05	0.90
65	21	4.53	14.67	16.15	45.8	50.6	101.2	32.4	32.0	1.03	1.07	0.97
66	22	4.99	13.44	13.47	47.8	47.9	95.8	26.9	28.1	0.86	1.01	0.85
67	22	4.85	13.82	14.24	47.9	49.4	98.8	28.5	28.9	0.92	1.04	0.88
68	22	5.45	12.89	11.83	51.1	46.9	93.8	23.7	25.2	0.76	0.99	0.77
69	20	5.14	13.07	12.71	48.0	46.7	93.4	25.4	27.2	0.82	0.98	0.83
70	20	5.23	16.17	15.46	44.7	42.7	85.5	30.9	36.2	0.99	0.90	1.11
71	23	4.83	16.74	17.33	46.5	48.2	96.3	34.7	36.0	1.10	1.01	1.08
72	20	4.92	14.03	14.26	40.7	41.4	82.7	28.5	34.5	0.92	0.88	1.05
73	30	4.68	18.00	19.23	51.6	55.1	110.3	38.5	34.9	1.22	1.15	1.06
74	22	4.68	14.90	15.92	46.0	49.2	98.3	31.8	32.4	1.02	1.04	0.99
75	21	5.08	18.70	18.41	51.0	50.2	100.4	36.8	36.7	1.18	1.06	1.11
76	21	4.87	15.39	15.80	45.1	46.3	92.6	31.6	34.1	1.02	0.98	1.04
77	20	5.17	14.56	14.08	48.8	47.2	94.4	28.2	29.8	0.90	1.00	0.90
78	22	4.86	15.02	15.45	45.0	46.3	92.6	30.9	33.4	0.99	0.98	1.01
79	20	5.15	15.14	14.70	48.5	47.1	94.2	29.4	31.2	0.94	0.99	0.95
80	21	4.61	14.13	15.33	44.4	48.9	96.3	30.7	31.8	0.99	0.98	0.97
81	20	4.27	13.63	15.96	49.3	57.7	115.5	31.9	27.6	1.02	1.22	0.84
82	21	4.43	13.92	15.71	48.7	55.0	109.9	31.4	28.6	1.00	1.15	0.86
83	21	4.90	15.90	16.22	48.8	50.0	99.6	32.4	32.6	1.04	1.05	0.99
84	25	4.94	15.39	15.58	46.9	47.5	94.9	31.2	32.8	1.00	1.00	1.00
85	22	4.99	14.44	14.47	47.4	47.5	94.9	28.9	30.5	0.93	1.00	0.93
86	26	5.05	14.44	14.30	48.0	47.5	95.0	28.6	30.1	0.92	1.00	0.92
87	22	5.39	17.34	16.09	53.8	49.9	99.8	32.2	32.2	1.03	1.06	0.97
88	26	4.32	14.90	17.25	45.7	52.9	105.8	34.5	32.6	1.12	1.13	0.99
89	23	5.19	13.72	13.22	49.0	47.2	94.4	26.4	28.0	0.85	0.99	0.85
90	26	5.05	14.03	13.89	44.4	44.0	87.9	27.8	31.6	0.89	0.93	0.96
91	24	4.78	14.67	15.35	44.7	46.8	93.5	30.7	32.8	0.98	0.98	1.00
92	28	4.95	14.90	15.05	43.5	44.0	87.9	30.0	34.3	0.97	0.93	1.04
93	29	4.85	13.82	14.25	49.0	50.5	101.0	28.5	28.2	0.92	1.06	0.86
94	22	4.86	14.56	14.98	45.0	46.4	92.6	30.0	32.4	0.96	0.98	0.98
95	26	4.58	15.02	16.40	47.4	51.7	103.5	32.8	31.7	1.04	1.09	0.96
96	21	5.11	16.02	15.68	48.2	47.2	94.3	31.4	33.2	1.01	1.00	1.01
97	21	5.35	16.59	15.51	47.9	44.8	89.5	31.0	34.6	0.99	0.94	1.05
98	27	4.61	14.67	15.91	44.4	48.2	96.3	31.8	33.0	1.02	1.02	1.00
99	23	5.23	14.56	13.91	49.5	47.3	94.6	27.8	29.4	0.89	0.99	0.89
100	25	5.19	16.31	15.71	46.5	44.8	89.6	31.4	35.1	1.01	0.94	1.07
Mean		4.84	15.12	15.71	46.5	48.3	96.5	31.4	32.4	1.01	1.02	0.99

lated a mean corpuscular hemoglobin of 32.4 for his series of 40 healthy men. The criticism of his method of hemoglobin determination has already been mentioned.

THE QUESTION OF GEOGRAPHIC VARIATIONS

The comparatively wide differences which have been found to exist in hemoglobin and erythrocyte count have frequently raised the question of possible geographic variations other than the well-recognized increase with altitude.

Parjono, Radsma and Joenoes[25] compiled data on blood iron and oxygen capacity in an effort to find some correlation between the amount of hemoglobin and geographic latitude. The data used were found insufficient to establish any clear relationship.

In Tables II and III are listed erythrocyte and hemoglobin determinations from various geographic localities. From these figures it is apparent that any correlation on the basis of geographic locality is very difficult, since in several cases, investigators working in the same locality have secured widely differing results. There are, however, few striking differences.

Many other factors than geographic location are doubtless existent as, for example, in the case of the difference found between poorer natives and the better-situated students and native doctors in the Java group of Parjono, et al. For this particular difference these authors suggest the prevalence of intestinal worms among the poorer classes as a possible explanation.

Orias[23] found a definite difference between common soldiers, officers and students in Buenos Aires (Table III). He concludes that social condition is an important factor in hemoglobin level. Horneffer also noted this difference, finding 16.25 gm. as the mean value for 20 students and 15.80 gm. as the mean for 20 soldiers.

Price-Jones suggests chronic carbon monoxide poisoning from motor cars, with an accompanying compensatory increase in hemoglobin and red blood cells to account for the difference in hemoglobin content which was found by him in London and Boston. This explanation is claimed by Jenkins and Don to have been invalidated by observations made on mechanics. These investigators find an even higher mean hemoglobin value for individuals from various localities in England than Price-Jones found in Americans.

The possibility of a difference in hemoglobin level between smokers and nonsmokers due to carbon monoxide has also been investigated,[32a] but no significant difference was found.

Some significant differences due to technic would seem inevitable in any list of investigations as large as those quoted in Tables II and III. Further sampling of the normal population on an extensive scale is needed to clarify the question of normal standards for general use.

SUMMARY

1. Careful observations made on 100 healthy men between the ages of twenty and thirty in Kansas show a mean erythrocyte count of 4.84 million, 15.12 gm. of hemoglobin per 100 c.c. of blood and 46.5 c.c. of packed cells per 100 c.c. of blood.

2. When a hemoglobin coefficient of 15.59 gm. and a volume coefficient of 47.4 c.c. are employed as 100 per cent standards, the color, volume and saturation indices are approximately 1.

3. Calculations from the above data give the following average figures: for mean corpuscular volume, 96.5 cubic micra; for mean corpuscular hemoglobin, 31.4 micromicrograms; for mean corpuscular hemoglobin concentration, 32.4 per cent.

4. Comparison of erythrocyte and hemoglobin values found by investigators in widely separated areas shows no apparent relation to geographic location. Technical variations may be one of the important factors in observed differences.

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WUCHERERIA (FILARIA) BANCROFTI INFECTION IN MAN WITH AN UNUSUAL HISTORY*

CASE REPORT

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THE occurrence of Wuchereria infection in a person who has remained within the vicinity of the District of Columbia all of his life is so unusual that we feel justified in reporting it.

We have not been able to discover all the links in the chain of circumstances leading to the infection. This, together with the usually accepted epidemiology of filariasis, while incidentally serving to remind the general practitioner of its possible occurrence in this part of the country, also serves to focus more interest of the entomologist on the way or ways in which the patient may have become infected. From an epidemiologic standpoint we thought of four possibilities: (1) a wider general distribution of species of mosquitoes generally known to serve as vector for this disease; (2) a special means of distribution by these same mosquitoes laying their eggs in the few centimeters of water frequently found in the bottom of the lifeboats on freighters coming from endemic ports with a crew, some of which may be carriers; the larvae from these eggs become mosquitoes in time to bite the crew and they, after the usual incubation period, infect persons in any subsequent port; (3) the common mosquito of this vicinity, which may assume the rôle of vector when in contact with carriers from an endemic area; (4)

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and lastly, some species of tick, which may serve as vectors. The last possibility is of interest chiefly because *Filaria perstans*, an organism very much like *Wuchereria bancrofti*, except that it does not have a sheath, is transmitted by a certain species of tick, and recently we have had an increase in tick (Eastern type of Rocky Mountain spotted) fever in this vicinity.

CASE REPORT

X. Y., a negro janitor, aged twenty-nine years, was admitted to Freedman's Hospital, May 15, 1933, with a chief complaint of progressive enlargement of the scrotum and right leg and foot with several areas of suppuration from the scrotal sac, one on the right buttock, and glandular enlargement and ulceration of the left axillary region.

The past history is important in that the patient has lived in Washington all of his life and has never travelled more than one hundred miles from Washington, and when he did leave the city, it was only for a short time, generally on fishing trips.

He has had measles, mumps, whooping cough, and eczema during childhood, but has otherwise enjoyed good health up to the beginning of the present illness. His appetite has remained good. He has used alcohol and tobacco in moderation, but no narcotics.

The only contacts which he can recall with the tropics or subtropical zones are: prior to the present illness he had worked as janitor for a family of Filipinos, then living in Washington; and his family at various times received packages, some of which contained plants, from relatives in Mississippi. The family history is essentially negative except for an almost complete progressive blindness in his father (cataract).

Present Illness.—About five years ago the patient, without any previous chill, fever or other symptoms of illness, began to have slight pains in the umbilical region. This continued for some time. Shortly after this he observed that his scrotum was enlarging. This continued, and about two weeks after the enlargement was noticed, two small areas of ulceration were observed where the scrotal walls rubbed against the thighs.

At this time his wife left him, accusing him of having some type of venereal disease.

He was treated by a private physician for about one year with various local and internal medications, but when the scrotum continued to enlarge, he was hospitalized for ten weeks at Gallinger Municipal Hospital. There, various studies and treatments were performed without any check of the progressive enlargement of the scrotum. They did not find microfilariae in the blood. His general condition of health was good up to the time of admission to Gallinger Hospital.

After leaving the hospital, he did not return to work because the size of the scrotum and the several areas of ulceration made walking more difficult.

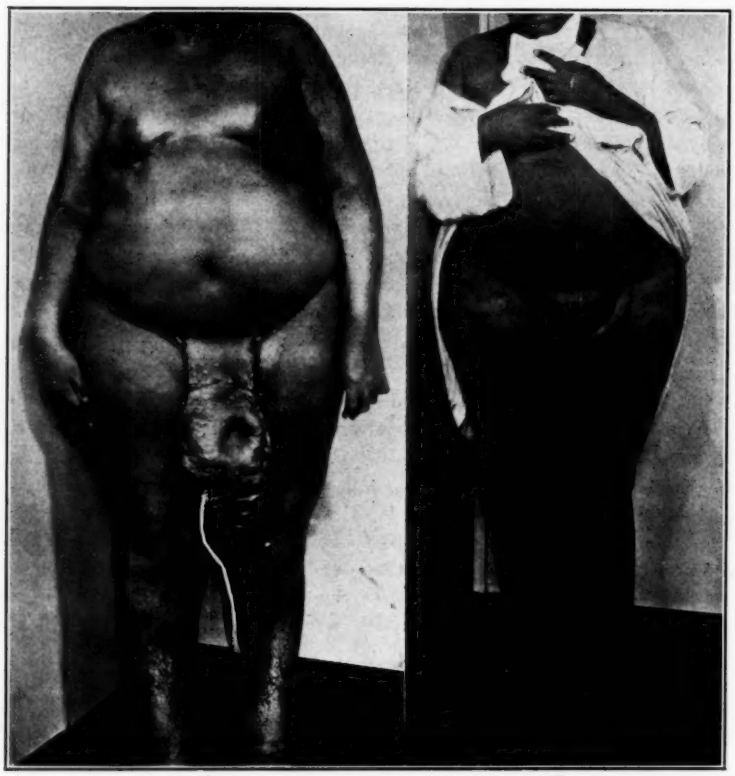
For the last four years he has not worked nor received any systematic medical treatment. During this time the scrotum has continued to enlarge. Areas of suppuration have developed over the scrotum and areas of excoriation over the inner surface of the thighs where they rub against the scrotal wall. Glandular enlargement developed in the axillae with subsequent suppuration of the left axilla. About this time a small ulceration developed over the right buttock and about two years ago the right leg and foot began to enlarge and to become progressively rough.

In the light of the above progress he was advised by a member of the staff of Freedman's Hospital to come into the hospital for study. This he decided to do and was admitted May 15, 1933.

Physical Examination.—The general examination revealed a very obese negro male, weighing more than 350 pounds, without any evidence of marked discomfort or illness, who was in a jovial mood. The head and neck were normal. The chest and upper extremities, except for some glandular enlargement in each axilla and slight suppuration of the left axilla, were normal. The abdomen showed abundant adipose tissue.

Genital examination revealed a very greatly enlarged scrotum, appearing to weigh about 25 pounds. It was deeply pigmented here and there, nodular and hard in places. Other

areas reflected light due to the drying of serum which had oozed from its surface, while still others presented deep sinuses covered by a purulent exudate. Where the folds of pendulous abdominal fat met the fat on the anterior surface of the thigh on either side, the valleys of the inguinal regions presented ulcerated surfaces penetrated by tunneled sinuses. These showed a marked tendency to granulation. While the penis was completely invaginated within the overgrowth of scrotal tissue, there seemed to be a marked proliferation of prepubic tissue, which was unusually pendulous. There were areas of excoriation over the inner surface of each thigh, where the very much enlarged scrotum rubbed against them. The right buttock showed a small area of suppuration. The left leg and foot, except for the marked dryness and scales, were apparently normal. The right leg was larger around by two inches at the calf and knee than the normal leg. The right leg and foot showed scaling and pigmenta-



A B

Fig. 1.—A, Before treatment. Measurements: Left, thigh 32 inches; knee 22 inches; calf 20¼ inches; ankle 14½ inches. Right, thigh 32½ inches; knee 24 inches; calf 22 inches; ankle 14 inches. B, After treatment.

tion together with a few shining areas in which serum appeared to be oozing. There was no definite ulceration of either limb.

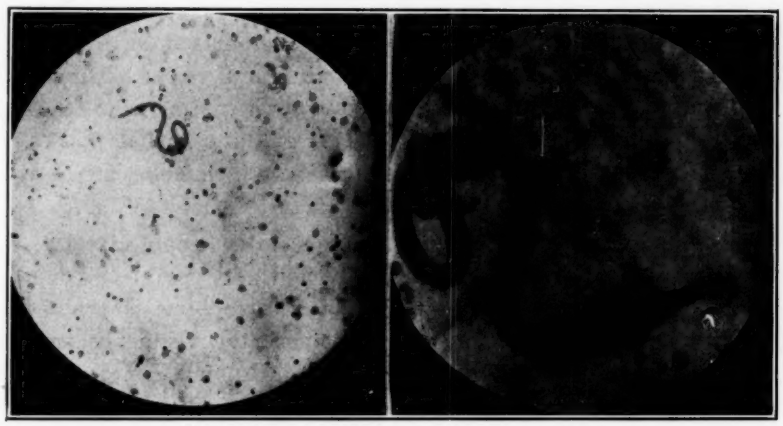
A clinical diagnosis of elephantiasis was made by the staff.

Laboratory Examination.—Several thick smears of blood taken during the night, one at 9:15 P.M. and another at 10:30 P.M. on separate nights, showed microfilaria when stained by the Giemsa method. The coagulation time was five minutes. Red blood corpuscles 4,670,000; white blood cells 14,000. Differential: Small lymphocytes 36 per cent, large lymphocytes 25 per cent, large mononuclears 8 per cent, transitionals 6 per cent, polymorphonuclear neutrophiles 20 per cent, eosinophiles 5 per cent. Blood Wassermann was negative. Urine showed no microfilariae, a few epithelial cells, and a positive indican test; otherwise nothing significant.

Stools were free from parasites, ova or larvae. Bacteriologic culture from several of the areas of ulceration and from some of the deep tissue removed at operation showed various micrococci, *Staphylococcus albus*, a nonhemolytic streptococcus, which fermented mannite and was considered to be *Streptococcus fecalis;* from one area there was a diphtheroid organism. The condition of the patient while in the hospital was uneventful. The earlier treatment consisted primarily of Sitz bath and mild local antiseptics in an attempt to clear up most of the secondary infection.

Attempts were made on two occasions to drain fluid from the enlarged scrotum. Several punctures with various sized needles failed to locate any definite pockets of fluid. We interpreted the enlarged scrotum to be due to chronic edema, and proliferation of the subcutaneous connective tissue secondary to this edema, the pathologic basis for which is the blocking of the lymph circulation.

The patient was sufficiently clear of secondary infection by June 2, 1933, to warrant an operation. On June 2, 1933, under general ether anesthesia, the enlarged scrotum was removed together with some of the inguinal lymphatics. Also an extensive section of the prepubic redundancy was removed, effecting complete recovery of the penis. It was found that the skin which covered the penis was not thickened, but only evaginated, due to the weight of the involved scrotum. The testicles were found to be normal in every respect. They were stripped



A B

Fig. 2.—A, Shows a low-power magnification of the Microfilaria stained with hematoxylin as described above. B, Shows the same Microfilaria under a higher magnification.

The Technic of Preparation Was as Follows.—Slides were made from the centrifuged specimens of citrated blood, which had also been dehemoglobinized by distilled water. The residue was used for the smear. The smear was fixed in a solution containing equal parts of alcohol and ether for five minutes; stained with warm hematoxylin for eight minutes, decolorized for two seconds in 80 per cent alcohol to which had been added a few drops of hydrochloric acid and dried for examination.

of all of the proliferative tissue and covered in a new sac by joining the healthy skin of the perineum and inner side of the thigh to the normal skin covering the penis. For several weeks the penis was visible. Erection was noted on many occasions. Failure to remove sufficient subcutaneous tissue in the prepubic region encouraged the return of redundancy with resulting invagination of the penis. The patient had an uneventful convalescence from the operation. He was discharged from the hospital July 7, 1933, markedly improved, with instructions to return at intervals to the out-patient department for observation and further treatment.

DISCUSSION

In view of the nodular swellings under the arms and elsewhere, we were compelled to consider a probable onchocerciasis, a rare disease due to Onchocerca volvulus, a member of filaridae family, and transmitted by some gnat of the Simulidae family.

The geographic distribution of the onchocerciasis, as well as the history of this case, made that solution highly improbable from the beginning, and the microscopic study of the microfilaria definitely ruled it out.

Clinically the case was one of elephantiasis. We therefore had to consider the etiology from three standpoints: infection with *Wuchereria bancrofti* and a secondary invader which some believe to be primarily responsible for the ulceration and much of the lymphatic blockage or elephantiasis of a nonparasitic type resembling the cases reported by Gager,[1] and others. While the works of O'Connor and Hulse,[2] and O'Connor[3] seem to show that elephantiasis in Wuchereria infection may occur without secondary infection, we believe that this case is one in which the secondary infection by the streptococci and staphylococci is an important secondary etiologic factor in producing the syndrome.

The question of where and by what means the patient received the infection seems to be the most important consideration here.

Wuchereria bancrofti infection, while common in various areas throughout the tropics and subtropical belts, is most important as a United States menace because of its prevalence in the Islands and ports of the Caribbeans and its endemicity in Charleston, South Carolina. Man is the only proved intermediate host harboring the adult worms and the sexual phases, and the mosquito is the only proved definitive host in which complete development may take place, and is thus the vector of interest.

Since we have evidence that man cannot infect man directly, but that the microfilariae must undergo a certain period and complete certain stages of development in the mosquito before the infective form is produced and is thus ready to be transmitted to another person, the vector becomes the important problem here. There are at least four known genera of the order Diptera in which the complete development of the *Wuchereria bancrofti* is known to be possible: viz., Aedes, Anopheles, Culex, and Mansonia. There are several species under each of these genera in which the complete development of Wuchereria is known to be possible, but for all practical purposes in the United States only three species are important as vectors. They are *Culex fatigans,* common in the West Indies and South Carolina; *Anopheles (Nyssorhynchus) albimanus,* common in the Caribbean; and *Aedes variegatus,* common in the Pacific Islands. While such species as *Anopheles costalis* are very efficient vectors, a geographic consideration of its habitat, primarily confined to the West Coast of Africa, the small amount of travel and trade between those ports and North America and the long distance make it a vector of almost negligible consideration in this case. For many of the same reasons, *Aedes variegatus* becomes less likely as a vector in this case. Using the above methods of reasoning, we feel that the frequent contact between Baltimore and the Caribbean and Charleston, South Carolina, makes the *Culex fatigans* or *Anopheles albimanus* the most likely vector in this case.

The question of this man as a carrier and a menace is worthy of mention only to rule out the danger. As pointed out by Manson-Bahr,[4] when fewer than one microfilaria were present in 2 c.c. of the patient's blood, the appro-

priate mosquito frequently failed to acquire infection. In this case, there was about one microfilaria per 3 c.c. of blood as averaged by our concentration method before operation and about one microfilaria per 5 c.c. of blood now. The usually accepted vectors are infrequent in this locality. We also know that some of the microfilariae are destroyed during their stay in the mosquito.

These facts together with the fact that both a male and a female must become lodged in the same area in man and then grow and develop their reproductive organs preparatory to bringing about a fecundated adult female parent before multiplication can take place, make this case insignificant as a danger. However, if the patient were more heavily infected, he would be a potentially dangerous carrier.

There is no accepted medication. Many clinics in the tropics report the trial and failure of various intravenous medications. I was impressed with the reports of success still claimed by the staff of the Municipal Hospital of Fredericksted, St. Croix, Virgin Islands, while visiting there during the summer of 1932. The other hospitals in various parts of the Caribbean did not report such results. While the surgical treatment selected in this case is primarily palliative and of no unusual significance, untold individual aid has been rendered this patient.

SUMMARY AND CONCLUSION

Wuchereria bancrofti infection may occur in persons living in the temperate zones who have not had any obvious contact with the commonly accepted vector. Such a case is here reported.

We wish to express our appreciation to Doctors William H. Taliaferro and Clay G. Huff of the Department of Parasitology, University of Chicago, for their opinion in regard to this species of Microfilaria, and to Dr. Porter B. Lennox of the Department of Pathology, School of Medicine, Howard University, for taking and developing the photomicrographs.

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THE TOXIN OF BACILLUS PROTEUS*

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THE toxic properties of cultures of proteus isolated from various sources has been the subject of considerable study since the time of Hauser's[1] original description of the type organism of the proteus group when he demonstrated its pathologenicity for animals.

That both solutions of the dried, sterile, powdered organisms and filtrates from cultures have definitely toxic effects has been repeatedly demonstrated, and Bengston[2] in summarizing the results of the work in this field states that "*B. proteus vulgaris* produces a weak soluble toxin, as compared with tetanus and diphtheria."

However, while agglutinins have been demonstrated in high dilutions following immunization and in infections with the organism, and while a relative immunity to the usual fatal doses has been produced, there seems to have been only one demonstration of an antitoxin. Herter and Ten Broeck[3] were unable to establish immunity to the toxic extracts of the powdered organisms.

Isabolinski and Judenitsch[4] succeeded in preparing two culture filtrates containing immunizing antibodies of different degrees of effectiveness. The cultures were obtained from patients suffering from typhus fever and were grown on agar plates and in broth. The "H" form contained more immunizing substances than the "O" form, as shown on rabbits receiving lethal doses of the original culture.

I have investigated the subject of antitoxin production in rabbits and attempted to form some idea of the relative toxicity of the filtrates for man.

The strain of proteus employed was isolated from catheterized bladder and ureteral urine from a girl, twenty years old, with uncomplicated pyelitis due to proteus.

The organism was a small gram-negative rod, motile in young cultures, gradually losing motility after the first twenty-four hours. On agar it spread rapidly over the plates forming a thin gray film, covering the plate from four to five streaks in twenty-four hours. It grew more rapidly on blood agar than plain. It liquefied gelatin, naptiform in twenty-four hours, and complete in from three to four days at room temperature. Litmus milk was changed to acid and coagulated in from two to three days, peptonized and alkaline in five days. Nitrites were formed. No indol was produced. Acid and gas are formed in dextrose, galactose, levulose and saccharose (after three days). No acid or gas was produced on lactose, maltose, raffinose, arabinose, mannitol, dulcitol, dextrin, glycerol, salicin, sorbitol and inulin. Sugar mediums were prepared by adding 5 per cent sugar to broth containing 1 per cent Liebig's meat extract 5 per cent Witte's peptone, and sodium chloride 5 per cent.

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Plain broth mediums, as used in fermentation tests, titrated to P_H 6.0 were used for growing cultures in preparation of the filtrates. Cultures were incubated from one to eleven days at 37° C. Each culture was checked for contamination before filtering. Berkefeld "N" filters were used for the first filtration; the filtrate was then passed through "W" filters and tested for sterility by both aerobic and anaerobic methods, culturing on blood agar slants.

EFFECT UPON RABBITS

The rabbits used were young and weighed between 1,500 and 2,000 gm., averaging about 1,700 gm. The filtrate was injected, unless otherwise specified, into the marginal ear vein.

Ten cubic centimeters of the filtrate of a one-day culture caused no apparent ill effect. Ten cubic centimeters of the filtrate of a three-day culture caused death in less than twenty-four hours. The same dose of the filtrate of a five-day culture failed to kill another rabbit but produced severe illness with diarrhea and moderate loss of weight. Five cubic centimeters of the filtrate of an eight-day culture caused death in less than twenty-four hours and in 6 rabbits all doses above this were fatal in less than twenty-four hours. Doses

TABLE I

THE EFFECT UPON RABBITS OF FILTRATES OF CULTURE GROWN FOR VARYING LENGTHS OF TIME WITH VARIATIONS IN THE DOSAGE. INTRAVENOUS ADMINISTRATION

RABBIT	DOSE IN C.C.	AGE OF CULTURE	RESULT
1	11	Broth control	No ill effects.
2	10	1 day	No ill effects.
3	10	3 days	Died in less than 18 hours. Heart's blood sterile. This rabbit had received 10 c.c. of broth control two days before without apparent ill effects.
4	10	5 days	Diarrhea for four days, loss of 250 gm. Recovery.
5	5	8 days	Died in less than 18 hours.
6	9	11 days	Very ill next day. Emaciation with loss of considerable weight. Died in 16 days.
7	5	11 days	Died in less than 18 hours.
8	10	11 days	Died in less than 18 hours.
9	10	11 days	Died in less than 18 hours.
10	10	11 days	Died in less than 18 hours.
11	10	Broth control	No ill effects.
12	10	Broth control	No ill effects.
13	1½	11 days	Died in two days; 13 c.c. of blood had been withdrawn 2 days before without apparent ill effects.
14	¾	11 days	Died in 24 hours; 13 c.c. of blood had been withdrawn 2 days before without apparent ill effects.
15	2	11 days (3 mo. old)	Lost 330 gm. in 4 days. Diarrhea was present on the first day and more marked on the second. Lost 610 gm. in 7 days.
16	2	11 days (3 mo. old)	Found dead in less than 18 hours.

of ¾ to 2 c.c. of a three-month-old filtrate killed 3 rabbits and produced severe illness in another. Doses of ¾ c.c. and 1½ c.c. killed 2 rabbits from which 10 c.c. of heart blood had been withdrawn two and nine days before, respectively.

One rabbit recovered from intramuscular injection of 5 c.c. of toxin which, when injected intravenously, killed rabbits of the same weight when administered in doses of less than 2 c.c. The rabbit injected intramuscularly, however, was sick and lost 20 per cent of its weight.

To determine the toxin production in gelatin, cultures were grown for six weeks at room temperature, diluted with salt solution in proportion 1:4 and filtered as described for broth filtrates; 1½ c.c. of this dilution killed a rabbit in less than twenty-four hours. One cubic centimeter caused profound illness, with loss of 29 per cent of weight in three days. Control gelatin produced no ill effects.

The results (Table I) demonstrate that less than 5 c.c. of filtrate from the eleven-day broth is usually a fatal dose for a rabbit weighing between 1,500 and 2,000 gm. Doses of 2 c.c. or less caused either death or profound illness. Doses of 10 c.c. always killed in less than twenty-four hours.

TOXIC EFFECTS

The most characteristic reaction to the filtrate was diarrhea, the entire contents of the small and large intestines being liquid, with marked distention. Microscopically there was a marked infiltration of the submucosa with leucocytes and lymphocytes, also hyperemia. The mucosa showed areas of sloughing and necrosis of the tips of the villi (postmortem). The Peyer's patches were frequently enlarged.

The kidneys were dark purplish red and showed areas of hemorrhagic infiltration with cloudy swelling of the cortex.

The liver was somewhat enlarged, but usually it showed no marked microscopic changes.

The heart was usually dilated and filled with a blood clot, the muscle being thin and flabby. Microscopically there were small areas of parenchymatous degeneration.

Blood cultures were uniformly sterile; urine cultures showed no proteus; *B. coli* was present twice; and spore formers, once or twice.

ANTITOXIN PRODUCTION

Five normal rabbits were selected and their serums tested for ability to protect other rabbits. Five cubic centimeters of their serum were allowed to stand at room temperature with 10 c.c. of the toxin onehalf hour before injection intravenously. (Table II.)

Four of the 5 died quickly. The fifth survived after severe illness.

To 3 of the rabbits from which serum had been tested, 7 weekly doses of eleven-day filtrate, increasing from 0.2 c.c. to 6 c.c. were given intramuscularly without apparent ill effects or loss of weight except slight diarrhea for two

TABLE II

EFFECT OF INJECTION OF TOXIN FROM ELEVEN-DAY BROTH CULTURES WITH SERUM FROM NONIMMUNIZED RABBITS

SERUM FROM RABBIT NUMBER	C.C. OF SERUM	C.C. OF TOXIN	RESULT
1	10	10	Very weak; lay on side; temperature, 105° F. Loss of 680 gm. in 14 days. Recovery.
2	4½	9	Died in 48 hours.
3	5	12	Found dead in less than 18 hours.
4	4	7½	Found dead in less than 18 hours.
5	5	11	Died with marked emaciation in 8 days.

days in one, following the fourth dose. The fourth rabbit was given similar increasing doses of broth.

Eleven days after the last dose, the serum was tested again. (Table III.)

TABLE III

EFFECTS OF INJECTION OF ELEVEN-DAY TOXIN WITH SERUM FROM RABBITS WHICH HAD BEEN IMMUNIZED BY SEVEN INTRAMUSCULAR WEEKLY DOSES OF TOXIN, THE CONTROL RECEIVING THE EQUIVALENT DOSE OF BROTH

SERUM FROM RABBITS	C.C. OF SERUM	C.C. OF TOXIN	RESULT
Immune, A	4	10	Slight diarrhea next day. Lost 200 gm. in 5 days. Well. Never weak.
Immune, B	4	10	Died in less than 18 hours.
Immune, B	4	10	Lost 260 gm. in 3 days. Snuffles appeared on the third day. Recovery.
Immune, C	4	10	Slight diarrhea. Lost 240 gm. in 3 days. Not very ill. Began to gain on third day. 1590 gm. on fifth day.
Control	4	10	Died in less than 18 hours.
Control	4	10	Died on third day, severe diarrhea.

In 3 rabbits more than three times the lethal amount of toxin was neutralized by 4 c.c. of immune serum. In one case 4 c.c. of immune serum failed to protect, but a second rabbit, after receiving the same amount of this antiserum and filtrate showed only moderate loss of weight with slight diarrhea and recovered.

Four cubic centimeters of the serum from the rabbit injected with broth failed to protect in 2 rabbits, one dying in twenty-four hours, the other in three days.

SKIN TESTS

The results of intradermal tests on rabbits show first that susceptibility to the filtrate is comparatively low. However, of 13 rabbits tested, all but one reacted positively to the undiluted toxin, and in this one the reaction area was almost up to the 1 cm. diameter taken as a minimum standard for a positive reaction.

Seven of the 13 rabbits were positive in dilution of 1:10, the reaction being less than 2 cm. in diameter in all but one.

Two of the 4 rabbits, given increasing intramuscular injections of the filtrates weekly for six weeks, reacted negatively to undiluted toxin after

having been positive to 1:10 dilution before immunization. The other two showed definite diminution in the intensity of the skin reaction.

Two rabbits which had been given transient bladder infections with the organism two months before reacted positively to a 1:10 dilution in the skin test.

To test the toxicity of the filtrate for man, preparations as described were diluted with normal salt solution, and the reaction of the skin to intradermal injections of 0.1 c.c. of various dilutions was observed eighteen and twenty-four hours later. A reaction area measuring 1×1 cm. or more, from eighteen to twenty-four hours after injection was considered a positive test.

In susceptible persons, redness appeared at the site of injection shortly after the test was made, fading in an hour or less. About four or six hours later pinkness the size of the welt was present and gradually increased in size and intensity to its maximum which might be reached in six or eighteen hours; it usually began to fade after about twenty-four hours and disappeared in from forty-eight to seventy-two hours. With well-marked reactions the color on the second day was brownish red fading to brown. If the reaction was fairly intense, from slight to moderate induration was present with elevation at the site of injection.

Freshly filtered and diluted toxin was found to give a positive test in 1 of 2 adults and 4 of 13 children (from three to six years old) in a dilution of 1:255,000. The reactions were faint. This solution was found to deteriorate rapidly, two weeks later giving a negative test in the previously positive persons.

Tests made at intervals during four and one-half months with 1:5,000 dilutions were positive. The last tests were about two-thirds the size of the first, indicating a slight deterioration at ice box temperature.

The solution, heated for one hour at 100° C., was used as control and was found to give only slight redness at site of injection, or negative tests. Test solution heated for two hours at 100° C. showed no redness at site of injection in twelve cases reacting positively to the unheated toxin and faint reactions a centimeter or less in diameter in two cases.

Strains of the organism kept on blood agar for three months retained the ability to produce a highly toxic filtrate. One such filtrate (eleven-day culture) gave a positive skin reaction in one and a negative skin test in 2 adults in dilution of 1:50,000. This filtrate two months later gave a negative test in the susceptible person in dilution 1:20,000, but was positive in dilution 1:10,000 and strongly positive in dilution 1:5,000. The skin test area was slightly larger in the case of this filtrate than in that from the freshly isolated strain.

CONCLUSIONS

B. proteus produces a toxin, the toxicity of the filtrate increasing with the age of the culture. Toxin production is about four times as great in gelatin as in peptone broth. The sterile toxin injected intravenously into rabbits causes diarrhea, loss of weight, and death.

Neutralization of this toxin in vitro by blood serum from rabbits immunized with it indicates that it is a soluble exotoxin capable of producing an antitoxin in rabbits.

Toxin solutions deteriorate on standing at ice box temperature and more rapidly at higher dilutions. The toxin is fairly stable in undiluted form. *B. proteus* cultures kept on artificial mediums for three months do not lose the ability to produce toxin.

Skin tests made with various dilutions of the toxin indicate that man is much more susceptible than the rabbit.

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PROFESSIONAL BUILDING

COCAINE POISONING AS INFLUENCED BY DIETS*

PRELIMINARY REPORT

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THE influence of diets, especially with alkalinizing and acidifying tendency, has been previously studied, particularly in connection with phosphorus poisoning. We have been interested in altering the acid-base balance of rabbits as a basis for studies in connection with seasonal diets and climatic changes. Hoping that we may find, in certain drugs, reagents that might help us in this study, we undertook feeding experiments upon rabbits, intended to alter their acid-base equilibrium, and then injected in these animals toxic doses of cocaine. The results have been sufficiently striking to make this preliminary report seem desirable.

We have used in these experiments various diets that will promote a prevalence of acids or bases in animals in the normal physiologic limits without causing any noticeable pathologic changes in them.

Forty-eight experiments were performed on thirty rabbits. These experiments were divided into three series of sixteen each. In each series, before the performance of an experiment, the animals were kept on certain diets for a period of three weeks. In the first series, the animals were kept on oatmeal

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and water; in the second series, on carrots; and, in the third, on oatmeal, water, and carrots. During the dieting the urine of the animals was tested daily for its reaction (Folin's method). From the second week on the urine of rabbits of the first series of experiments showed distinct and stable acid reaction (from 14 to 60); the urine of the animals of the second series was persistently alkaline; and that of the third series, slightly alkaline or neutral. In the third series of experiments, the same eight rabbits were used twice; the animals of the first and second series of experiments were changed from one diet to another. Ten rabbits withstood these experiments and were on both diets, and six succumbed, so that the latter had to be replaced by new animals.

The experiments consisted of injections of cocaine into the marginal ear vein of a rabbit. The injections were performed uniformly, the duration of

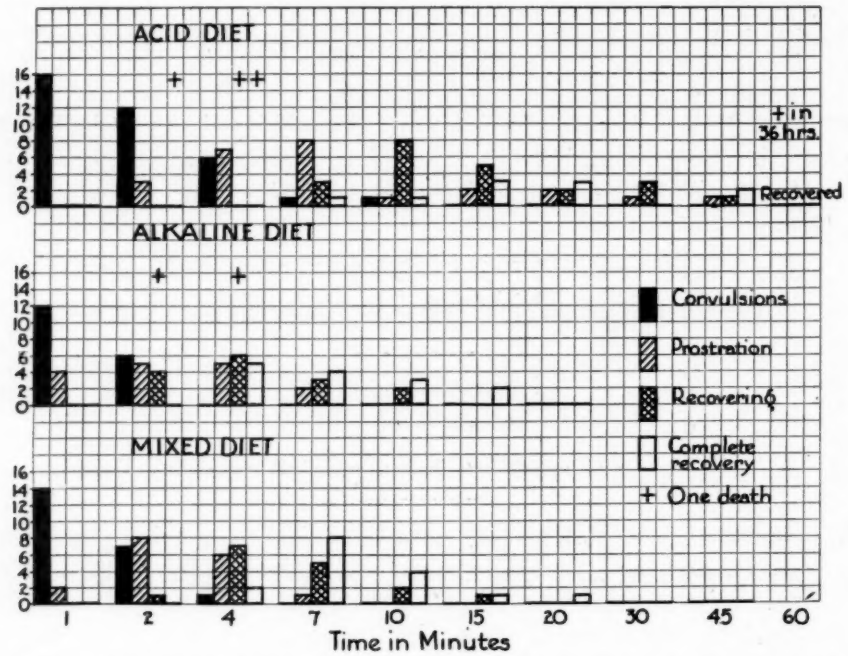

Chart 1.—The perpendicular line shows the number of rabbits. The columns on the horizontal lines are grouped by the time that elapsed from the moment of cocaine injection (1, 2, 4, 7, 10, 15, 20, 30, 45, and 60 minutes) and represent the degree of reactions (see chart) and number of animals. Each group of columns represents the same animals, reacting differently at various time intervals. The animals that died or recovered at certain times of observation are not shown in the following column.

injection being about 10 seconds. The dose of cocaine was 10 mg. per kilo. After injection, the animal was observed as long as symptoms were manifest. The degree of intoxication was recorded by checking the (1) death, (2) convulsions, (3) complete prostration without convulsions, (4) recovering (crawling, lifting head), and (5) recovery. Before the injection of cocaine a certain amount of blood was withdrawn from the ear vein and tested for its hydrogen ion concentration. The average P_H of the blood of the rabbits on acid diet was 7.301, on alkaline diet 7.426, and mixed diet 7.423, which showed that the rabbits in our experiments were within the limits of the normal acid-base balance.

The results are presented in Chart 1.

The described observations permit us to arrive at the following conclusions:

1. The animals on alkaline-forming diet and on mixed diet reacted to the injection of cocaine, more or less similarly; though the animals on the alkaline diet reacted more vigorously, since we recorded two deaths in this series.

2. The animals on acid-forming diet were definitely more sensitive to cocaine poisoning. Here were recorded four deaths; and, in general, the toxic effects of the cocaine were definitely greater and recovery was considerably delayed.

3. The observations presented suggest the investigation of a number of other powerful drugs in connection with their action upon the animal organism in various states of acid-base balance. Only then would it be permissible to speculate upon the reason for the difference noted.

4. It may be possible, that, in acute cocaine poisoning, the intravenous injection of alkali might be of benefit for the poisoned individual; and experiments upon animals, to test this question, are called for.

NASOPHARYNGEAL FLORA AND SOME REMARKS AS TO THEIR RELATION TO COMMON COLDS*

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THE common cold is generally defined as an acute or subacute infection of the mucosa of the nasopharynx, often invading the sinuses and occasionally the middle ear through the eustachian tube. At times, it may also extend through the trachea, larynx, and bronchial tree. Furthermore, it may leave a chronic condition locally or elsewhere.

Although the common cold is considered the mildest and least serious of all known common diseases, yet it is most frequent, annoying, and depressing; and it often results in the lowering of resistance of the individual, thus making him prey to more serious diseases. Generally speaking, no one is exempt from attacks of colds. Some have occasional attacks, others frequent attacks. One may have mild infection, while others have severe ones. In either case, it lessens the efficiency of the worker, resulting in great total loss economically.

A tremendous amount of experimental and research work is carried on concerning common colds, both in this country and abroad. Medical periodicals have published voluminous articles on etiology, pathology, and treatment of this condition. Unfortunately, it seems we are still wandering in the dark and very little has come to light which points to a definite conclusion about this common ailment.

In reviewing the literature on this subject, within the last few years, one

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finds that quite a few well-outlined and definite studies have been carried on by various workers. I will quote briefly the conclusions of some of the papers:

From their cultural studies of nose and throat of 105 adults and children, Webster and Hughes[1] isolated pneumococci in 80 per cent of cases, and out of 500 pneumococcic strains 97 per cent were serologically specific. Successive cultures from given carriers, with rare exceptions, had a constant serologic type and were similar in type of colonies, morphology, virulence for mice, and in other biologic characteristics. They conclude also that the incidence of pneumococci in all individuals studied undergoes seasonal variation paralleling that of coryza and sore throat in the same person. Third, some patients were pneumococcus-free; some were transient carriers; some periodic, and others chronic carriers. The data suggest that these differences are due to variations in the host resistance.

According to the observations made by Webster and Clow[2] of the association of pneumococci, *Hemophilus influenzae* and *Streptococcus hemolyticus* with coryza, pharyngitis, and sinusitis in man, they came to the conclusion that persons free of those organisms mentioned above are, in general, free of coryza, sore throats, influenza, and sinus diseases. Also they found that persons who are occasional or periodic carriers of these organisms may be negative on tests carried out during long healthy periods, but that they generally become positive during or following attacks and subsequently become negative again with returning health.

Third, their data suggest that persons who are chronic carriers show during these illnesses an increase in the number of organisms in the throat and wider distribution of these organisms in the air passages. These observations bring out the intimate relationship between pathogenes of upper respiratory diseases and their symptoms, but they do not disclose the actual nature of this relationship.

In their experimental studies of the common cold, Dochez, Shibley, and Mills[3] state that in both spontaneous and in experimentally induced colds in anthropoid apes, the most significant change that occurs is the increase of activity on the part of the potential pathogenes habitually present in the throat flora. Coincidentally, with the appearance of symptoms, they found that pneumococci, *Streptococcus hemolyticus* and Pfeiffer's bacillus developed in greatly increased numbers and spread over wide areas of nasopharyngeal membranes. These organisms become, after a time, conspicuous in the nose where they are seldom or never present under normal conditions. This has also been observed in human beings. Furthermore, they emphasize the fact that these organisms are secondary invaders and that the most significant factor in common cold pathogenesis seems to be their capacity to incite greater activity on the part of the more dangerous pathogenic organisms which infect the upper respiratory tract.

D. Thompson and R. Thompson,[4] in an extensive article, stress several points on common colds; that the common cold is not a definite single disease, but a group of diseases caused by several different species of organisms and other factors. According to their classification, colds are divided into three groups: (1) bacterial; (2) allergic; and (3) virus colds.

The bacterial flora of the healthy respiratory tract is plentiful and in great variety but has not been thoroughly worked out and classified. The streptococcus is the most common organism of any virulence generally found; next in frequency of occurrence are the pneumococci and *B. influenzae.*

The following are observations made in infants over a period of two years by Kneeland and Dawes.[5] In two severe winter outbreaks of respiratory infection, a parallel rise in carriers of pathogenic organisms was noted. The first autumn outbreak of colds seemed to provide the dissemination of pathogenic organisms. The relationship of colds to the more severe infections was roughly reciprocal. The peak of the carrier rate was reached in March. It seemed that *Hemophilus influenzae* was more predominant in the first year, and pneumococci, in the second year of the study.

Recent studies of filtrable viruses as etiologic factors of colds, by Dochez, Shibley and Mills[6] suggest that the contagious cold in human beings is caused by an invisible and unculturable, filtrable agent which, in all likelihood, belongs to the group of so-called submicroscopic viruses. Colds can be transmitted successfully, they state, from man to chimpanzee and from man to man by means of Berkefeld filtrates of nasal washings obtained from individuals suffering with spontaneous colds, more especially during the period of rising incidence of infection in the community. This experimental cold resembles in all respects those spontaneously contracted in the natural environment.

Cultivation of the virus of the common cold by Powell and Clowes[7] implies, according to their work, that 80 per cent of subjects used experimentally contracted inoculated colds. No subjects were used except those who had no history of colds for two months and showed records of two or three colds per year. They were inoculated during that period of the year when colds were not prevalent. This verified the conclusion of Dochez that the virus of the common cold is easily cultivated in an appropriately prepared chick embryo and is readily transmitted to human subjects.

Burkey and Freese[8] lay claim to isolating a gram-negative filtrable anaerobic organism in 92 out of 114 examinations of individuals in widely separated communities. Positive cultures were obtained from persons with or without colds both during the acute and the later stages of the disease. The strains which were cultivated were prevalent in normal throats as well as in the nasopharynges of persons suffering from colds at the time. More satisfactory results were obtained in filtering the nasopharyngeal washings through a Berkefeld V-filter. This series of examinations was too small to lead to definite conclusions. The results indicate, however, the presence of these organisms in material from the mouth as well as the pharynx.

In studies concerning the prevention and treatment of colds with vaccine, Murray[9] compared a group of 85 persons who were 100 per cent susceptible to colds with a group of uninoculated controls, within a period of six months covering the seasons of prevalence of colds. Figures and clinical experience with a mixed catarrhal vaccine in that clinic have shown that vaccine therapy offers as much, if not more, than any other preventive measure that has been tried against colds. It is well worth the while to try this type of preventive measure in persons susceptible to colds.

Three years of experience with stock vaccine in colds and in the prevention of respiratory disorders, according to one observer in the literature, indicated that the value of stock vaccines varies with the individual subjected to the therapy.[10]

The purpose of our study was:

(a) To isolate the organisms in the nose and throat of individuals having frequent colds, during the acute attack.

(b) To study in a like manner a number of individuals without colds, or relatively immune to colds.

(c) To carry through parallel cultures of these organisms in hormone broth and in the blood of the subject.

(d) To prepare vaccines from these growths found in the autogenous blood and to attempt the immunization of the subjects with these vaccines.

(e) To attempt, incidentally, to determine the effects of tonsillectomy on the frequency of colds.

These studies were carried out on patients visiting the Out-Patient Department of the Montefiore Hospital. Cultures were taken from 200 patients. From these, 100 were chosen as controls because they had had no colds for a long period of time. The other 100 patients gave histories of frequent colds throughout the year. A few of these complained of regular seasonal colds. In this series 82 were males and 118 females; 64 had had tonsillectomy; 136 had tonsils intact.

The culture technic was carried out as follows: The throat of each patient was rinsed with sterile salt solution. A cotton swab was then rubbed against the pharyngeal wall and a second swab was contaminated in the nostril. Special care was taken not to contaminate the swab by touching the floor of the mouth. These swabs were placed in nutrient broth, incubated for from 24 to 48 hours, and subcultured on blood agar plates. Parallel with these broth cultures, swabs treated as described above were placed in from 3 to 5 c.c. of fresh whole blood obtained from the patient's vein. After 24 hours of incubation, a drop of this blood was plated and the colonies resulting were identified.

Finally, vaccines were prepared from the organisms isolated in the autogenous blood cultures and the patients who were highly susceptible to colds were inoculated. The technic of preparation of the vaccine was as follows: A 48-hour growth of the organism isolated in the autogenous blood medium in each case and subcultured on hormone broth was treated chemically, utilizing saturated tricresol solution, giving a final dilution of 0.5 per cent tricresol in the vaccine. In 48 hours this was tested for sterility. The vaccine was administered in a starting dose of 1 ♏ twice a week and was progressively increased to a maximum of 15 ♏ twice a week. With this technic, the patient received the benefit of the activity of both the bacteria and its elaborated toxin in broth media. Patients who were highly susceptible to colds were inoculated with vaccines prepared according to the method just mentioned. The number of patients that received regular treatments thus far was only seventeen, and it is hardly fair to draw final conclusions. Ten

of seventeen showed relief from colds thus far, and the rest had marked improvement; the frequency and severity were markedly diminished. Final report will be given at the end of treatments administered to a hundred or more cases.

Results of the studies:

(a) The cultures in hormone broth generally showed a mixed growth of various organisms. Staphylococci, streptococci, pneumococci, *Micrococcus catarrhalis,* and unidentified gram-positive and gram-negative cocci and bacilli.

(b) Cultures in autogenous blood media generally showed a single organism, but occasionally more than one.

Incidence of various bacteria in broth culture and in autogenous blood cultures.

TABLE I

TWO HUNDRED CULTURES FROM NASOPHARYNGES IN AUTOGENOUS BLOOD

ORGANISM	NUMBER	PERCENTAGE
Staphylococci	126	63
Streptococci	78	39
Pneumococci	64	32
Micrococcus catarrhalis	8	4
Gram-positive bacilli	12	6
Gram-negative bacilli	4	2
Sterile	40	20

TABLE II

ONE HUNDRED CULTURES FROM NASOPHARYNGES OF PATIENTS FREE FROM COLDS

ORGANISM	BROTH MEDIUM	AUTOGENOUS BLOOD
Staphylococci	55	51
Streptococci	53	41
Pneumococci	52	38
Micrococcus catarrhalis	45	4
Gram-positive bacilli	10	4
Gram-negative bacilli	45	5
Sterile	0	10

TABLE III

ONE HUNDRED CULTURES FROM NASOPHARYNGES OF PATIENTS SUSCEPTIBLE TO COLDS

ORGANISM	BROTH MEDIUM	AUTOGENOUS BLOOD
Staphylococci	64	63
Streptococci	50	39
Pneumococci	38	31
Micrococcus catarrhalis	22	4
Gram-positive bacilli	11	6
Gram-negative bacilli	5	2
Sterile	0	21

Analysis: Tables II and III may be compared. The former applies to the 100 controls who were, as stated, free from colds. The latter is descriptive of the flora found in 100 patients who were susceptible to colds. The highest number of cultures showed staphylococci, considering the entire 200 cultures. Next in the same group we found streptococci and pneumococci to be most numerous, in the order named. It should be noted also that 20 per cent of the cultures carried out on autogenous blood remained sterile. From the comparative study of these tables, it is easily seen that those organisms grown on broth and unidentified failed to grow in the autogenous blood medium.

According to the theory propounded by Dr. Meyer Solis-Cohen and coworkers[11] an organism is pathogenic to the individual only if it is grown in his blood; and failure to do so indicates resistance in the individual to the organism. His findings, further, have been confirmed by others; notably Bull and Bartual,[12] Black, Fowler and Pierce,[13] Smiley,[14] and T. Matsunami and J. A. Kolmer.[15]

CONCLUSIONS

1. Individuals who are free from colds, and those who have frequent colds show no notable difference from each other in their nasopharyngeal bacterial flora.

2. The resistance of the individual is probably a more important factor in susceptibility to colds than is the nasopharyngeal bacterial flora.

3. Vaccines prepared from cultures of organisms of the nasopharyngeal flora on autogenous blood media (pathogen selective) appear to have given relief from the tendency to the common cold in 59 per cent of the cases.

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LABORATORY METHODS

A SIMPLIFIED MICRODETERMINATION OF CHOLESTEROL IN WHOLE BLOOD, SERUM, AND PLASMA*

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THE basis of all colorimetric methods for the determination of cholesterol is the Liebermann-Burchard reaction, which is conducted on an anhydrous chloroform solution of the blood cholesterol. Bloor[1] obtains this by extracting the cholesterol with hot alcohol ether, evaporating the solvent and dissolving the residue in chloroform. This method, especially in the micromodification of Sackett[2] is most widely used in clinical laboratories. Other methods dry the blood on plaster of Paris at 100° C. (Myers and Wardell[3]) or on filter paper in air (Ling[4] and Leiboff[5]) and extract with hot chloroform in special continuous extraction apparatus.

The use of heat occasionally produces a brownish off-color tinge in the unknown, which renders colorimetric comparison difficult. We therefore tried to determine if the extraction of the cholesterol may be effected at room temperature without the use of complicated extraction apparatus. Preliminary experiments showed that the cholesterol is quantitatively extracted by chloroform from blood dried on filter paper in two hours at room temperature. Therefore, the following procedure was adopted for the determination of cholesterol in blood.

PROCEDURE

On an ordinary 7 cm. filter paper, preferably ashless, deposit 0.2 c.c. of oxalated blood, serum, or plasma. Work the paper around until the liquid is distributed fairly evenly over the surface and then suspend it by means of a thread or wire strung through an unstained area in an incubator at from 35° to 40° C. After from twenty to thirty minutes the blood is completely dried. Roll the paper up compactly; fold into quarters and deposit it in the bottom of an ordinary 6 inch × 1 inch test tube. Into the tube, pipette 10 c.c. of chloroform; cork, and set aside for at least two hours. During this period the chloroform should completely cover the folded filter paper. In this manner a quantitative extraction of cholesterol from dried blood, serum, or plasma is effected without the use of heat, thus avoiding the occasional appearance of an off-color. Now, pipette 5 c.c. of the chloroform solution into an ordinary test tube. Into a similar tube place 5 c.c. of the working cholesterol standard.†

*From the Department of Laboratories, Israel Zion Hospital.
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†Stock Cholesterol Standard: Dissolve 1 gm. C.P. cholesterol in chloroform to make 100 c.c. of solution.

Working Cholesterol Standard: Dilute 5 c.c. of the stock cholesterol standard with chloroform to make 1000 c.c. of solution; 1 c.c. contains 0.05 mg. cholesterol. Both standards keep indefinitely in a refrigerator.

To each tube add 2 c.c. of acetic anhydride and 0.2 c.c. of concentrated sulphuric acid. Stopper, mix by inversion and allow to stand for fifteen minutes in a cool, dark place. Compare in the colorimeter.

Comparison:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 250 = \text{mg. cholesterol per 100 c.c. blood.}$$

Set unknown at 25 mm. and compare. The reading of the standard multiplied by ten gives the milligrams of cholesterol in 100 c.c. of whole blood, serum, or plasma.

This method is especially suited for use in clinical laboratories where the determination of cholesterol is conducted as part of a routine. The blood may be dried and allowed to extract with chloroform while the remainder of the routine is carried on. The actual working time per determination is from four to five minutes.

This procedure was conducted on 125 specimens of blood, serum, and plasma ranging in cholesterol content from 76 to 585 mg. per 100 c.c. and checked by the Bloor-Sackett method. In all cases, the results obtained agreed within 9 mg. per 100 c.c. with an average difference of 3.55 mg.

SUMMARY

Blood is dried in air on filter paper, extracted with chloroform at room temperature for at least two hours, and the Liebermann-Burchard reaction is conducted on the extract.

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EXAMINATION FOR TUBERCLE BACILLI*

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KOERTH and Hibbard[1] have recently reported a comparison of methods for staining tubercle bacilli, based on the use of three stains. They claimed better than 25 per cent advantage for the Spengler stain over the Ziehl-Neelsen method. The Schulte-Tigges method, though nearly as efficient as the Spengler by their test, was ruled out as more difficult and more subject to errors in technic.

We have examined one hundred sputums chosen at random, except that they had been reported negative or contained less than six bacilli with the Ziehl-Neelsen technic. Fifty were twenty-four-hour specimens, the other fifty being collections over a period of three days.

When the results were being tabulated, it was found that seventy-one patients were represented, covering all stages of the disease. A large number had never given a positive sputum by direct smear, nor had guinea-pig inoculations proved a diagnosis of tuberculosis on many of them.

A five-minute time standard was used. By "positive" is meant that six or more bacilli were found during this period. In a "negative," no bacilli were found during a five-minute search. If less than six bacilli were seen during the five-minute interval, the number is given.

The results are shown in the following table:

STAIN	NEGATIVE	POSITIVE, MORE THAN 6 BACILLI	LESS THAN 6 BACILLI†	NUMBER OF SLIDES SHOWING BACILLI
Ziehl-Neelsen	95	0	5	5
Spengler	85	10‡	5	15

†Not recognized as positive by some authorities.

‡Five of these positives were those specimens where less than six bacilli were found by the Ziehl-Neelsen method. The Spengler simply increased the number.

The conclusion is drawn in favor of the Spengler method. We have also found the method satisfactory in tissue staining.

A second study was made on the comparative value of two concentration methods, the hydrogen peroxide[2] and the antiformin method.[3]

Here, too, the series was short. Examinations were made of some twenty cases which had given negatives in from one examination to examinations covering several months. Some had never given a positive.

Except when guinea pigs were to be inoculated, the tests were made on sputum sterilized by autoclave. Controls of known positives, both sterile and unsterile, were used by both methods. Tubercle bacilli found in the glands

*From the Grasslands Hospital, Valhalla, New York.
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and spleen of an inoculated guinea pig proved that the organism remained viable after being subjected to the hydrogen peroxide treatment.

The two procedures checked closely, one more positive slide being found by the peroxide concentration than by the antiformin method. As this procedure was much less time consuming, making it possible to report the result of an examination on the day the specimen was received, it was chosen as our routine. In a group of two hundred specimens (all of which had been negative at the examination immediately preceding) treated by this method and centrifuged fifteen to thirty minutes at fairly high speed, we were able to report thirty-one positives and the presence of a few bacilli (less than six) in four other cases. This made thirty-five concentrations, or 17.5 per cent, showing organisms when from one to many previous examinations had been negative.

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HEMOGLOBIN AND BLOOD CELL RELATIONS AS DETERMINED BY IRON AND OXYGEN CAPACITY METHODS*

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IN A recent article Haden[3] has summarized the various methods employed for the determination of hemoglobin and the present state of knowledge regarding the normal hemoglobin content of blood. He points out a certain lack of agreement in the three largest series of determinations of hemoglobin in normal men and women. The oxygen capacity hemoglobin method of Van Slyke was the basis of comparison in the series quoted, namely, those of Osgood,[5] Wintrobe and Morell,[8] and Haden.[4] The absence of any large series of hemoglobin determinations in normal individuals in which iron determinations are used as the basic method is noted by Haden and also pointed out as desirable by Peters and Van Slyke.[6]

We are, accordingly, reporting hematologic studies on a number of patients and normal individuals of both sexes, in which the hemoglobin determinations were based both on the oxygen capacity and the iron content of blood.

Most of the individuals examined lived in or near St. Louis, although some were medical students who had spent the greater part of their lives in other localities in this country. Twenty-three normal men and thirteen normal women were studied. In addition observations were made on thirty patients ill with various conditions.

*From the Department of Internal Medicine, St. Louis University.
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Blood was drawn shortly before noon, with minimal stasis, into a large syringe from which it was delivered into a 25 c.c. volumetric flask containing exactly 3 c.c. of 1.6 per cent sodium oxalate solution. All work was done on the mixed dilution so obtained. A 1 c.c. to 200 c.c. dilution with Hayem's

TABLE I

HEMOGLOBIN AND CELL RELATIONS IN NORMAL MEN

DESIGNATION OF INDIVIDUAL	HEMOGLOBIN GRAMS PER 100 C.C. BLOOD				ERYTHROCYTE COUNT, MILLIONS PER C.MM.	HEMOGLOBIN COEFFICIENT	PIGMENT/CELL RATIO	HEMOGLOBIN PER CENT	COLOR INDEX	CELL VOLUME PER CENT	VOLUME INDEX	SATURATION INDEX
	OXYGEN CAPACITY	PROTEIN IRON	ACID HEMATIN	THREE-METHOD AVERAGE								
1	18.30	17.76	18.03	18.03	5.280	17.07	3.43	119	1.12	48.7	1.07	1.05
2	18.18	17.48	17.71	17.79	5.400	16.48	3.29	118	1.09	48.8	1.05	1.05
3	18.00	17.54	17.50	17.68	5.320	16.62	3.34	117	1.10			
4	17.68	17.75	17.28	17.57	5.920	14.83	2.97	116	0.98	48.0	0.94	1.05
5	17.60	17.61	17.10	17.44	5.420	16.11	3.24	115	1.06	46.6	1.00	1.06
6	17.10	17.10	17.70	17.30	5.480	15.78	3.16	114	1.04	49.5	1.05	0.99
7	17.65	17.30	16.88	17.28	5.600	15.42	3.09	114	1.12	47.5	0.98	1.04
8	17.30	16.90	17.25	17.15	4.910	17.45	3.49	113	1.22	47.5	1.12	1.03
9	17.10	16.89	16.63	16.87	5.380	15.69	3.13	111	1.03	46.2	1.00	1.04
10	17.00	16.82	16.59	16.80	5.270	15.93	3.19	111	1.05			
11	16.41	16.66	16.88	16.65	4.740	17.56	3.51	110	1.16	47.0	1.15	1.01
12	16.70	16.10	16.68	16.49	5.760	14.31	2.86	109	1.04	50.0	1.01	0.94
13	16.50	16.40	16.48	16.46	5.560	14.79	2.96	109	1.08	45.5	0.95	1.03
14	16.58	16.58	16.20	16.45	4.920	16.75	3.34	109	1.11			
15	16.48	16.20	16.38	16.35	4.780	17.11	3.42	108	1.13	44.1	1.09	1.04
16	16.54	16.21	15.75	16.17	5.100	15.84	3.17	107	1.05	45.8	1.04	1.01
17	16.80	15.92	15.53	16.08	5.200	15.45	3.09	106	1.02	46.6	0.99	1.07
18	16.04	16.06	15.47	15.86	5.060	15.67	3.14	105	1.04	45.4	1.04	1.00
19	15.67	15.84	15.80	15.77	5.020	15.70	3.14	104	1.04	43.8	1.02	1.02
20	15.60	15.51	16.11	15.74	5.226	15.06	3.02	104	0.99	44.0	0.98	1.01
21	15.58	15.58	15.96	15.71	5.360	14.65	2.93	104	0.97	46.1	1.00	0.97
22	15.58	15.00	15.10	15.23	5.500	13.84	2.77	101	0.95	45.5	0.97	0.96
23	15.25	15.00	15.10	15.12	5.560	13.60	2.72	100	0.94	44.9	0.94	0.96

TABLE II

HEMOGLOBIN AND CELL RELATIONS IN NORMAL WOMEN

DESIGNATION OF INDIVIDUAL	HEMOGLOBIN GRAMS PER 100 C.C. BLOOD				ERYTHROCYTE COUNT, MILLIONS PER C.MM.	HEMOGLOBIN COEFFICIENT	PIGMENT/CELL RATIO	HEMOGLOBIN PER CENT	COLOR INDEX	CELL VOLUME PER CENT	VOLUME INDEX	SATURATION INDEX
	OXYGEN CAPACITY	PROTEIN IRON	ACID HEMATIN	THREE-METHOD AVERAGE								
24	15.90	15.24	15.02	15.39	4.788	16.07	3.22	102.0	1.08	43.0	1.09	1.08
25	15.50	15.14	15.30	15.31	4.920	15.57	3.16	101.0	1.07	43.2	1.02	1.07
26	15.33	15.16	15.41	15.30	4.900	15.61	3.19	101.0	1.03	42.7	1.01	1.04
27	14.53	14.85	15.30	14.89	4.840	15.39	3.08	98.4	1.02	42.0	1.01	1.04
28	14.29	14.08	14.64	14.34	4.860	14.75	2.95	94.8	0.98	40.9	0.98	1.03
29	14.51	13.98	13.98	14.16	4.680	15.12	3.03	93.5	0.96	41.0	1.01	1.01
30	14.40	13.88	14.17	14.15	4.280	16.54	3.31	93.5	1.09	40.0	1.09	1.07
31	14.20	13.88	13.82	13.97	4.680	14.93	2.98	92.3	0.99	40.8	1.02	1.04
32	14.20	13.20	13.88	13.83	4.870	14.20	2.84	91.4	0.94	38.2	0.91	1.06
33	13.40	12.81	13.19	13.13	5.030	13.00	2.61	86.8	0.86	39.3	0.91	0.95
34	12.50	12.90	13.00	12.80	2.834	13.25	2.65	84.6	0.87	37.5	0.90	1.00
35	12.80	12.52	12.90	12.74	5.228	12.12	2.44	84.1	0.80	39.8	0.88	0.87
36	12.40	12.00	12.15	12.18	4.240	14.36	2.87	80.2	0.95	36.4	0.99	0.96

TABLE III

OBSERVATIONS ON PATIENTS

DESIGNATION OR DIAGNOSIS	HEMOGLOBIN GRAMS PER 100 C.C. BLOOD				ERYTHROCYTE COUNT, MILLIONS PER C.MM.	HEMOGLOBIN COEFFICIENT	PIGMENT/CELL RATIO	HEMOGLOBIN PER CENT	COLOR INDEX	CELL VOLUME PER CENT	VOLUME INDEX	SATURATION INDEX
	OXYGEN CAPACITY	PROTEIN IRON	ACID HEMATIN	THREE-METHOD AVERAGE								
72532 Familial Hemolytic Icterus	17.40	17.08	17.35	17.28	5.530	15.4	3.09	113.0	1.02	46.6	0.98	1.05
72442 Lethargic Encephalitis with Vomiting. Free HCl present	17.15	16.80	17.18	17.04	5.590	15.2	3.05	112.0	1.01	48.8	1.01	0.99
71874 Amenorrhea with Genital Hypoplasia. Depressive Psychosis	16.70	16.20	16.20	16.37	4.880	16.3	3.36	108.0	1.11	45.8	1.02	0.99
72317 Gastroenteritis	15.90	16.01	15.80	15.90	5.320	15.0	3.17	105.0	0.99	44.3	0.98	1.02
71963 Aortic Stenosis	15.85	15.85	15.00	15.57	5.084	15.2	3.08	102.0	1.00	45.4	1.03	0.97
71991 Duodenal Ulcer. Enteroptosis	15.02	15.23	15.23	15.16	5.620	13.5	2.70	110.0	0.89	44.3	0.92	0.97
70861 Epilepsy	14.50	14.81	14.59	14.63	4.540	16.0	3.24	96.6	1.06	42.0	1.08	0.99
72131 Glomerular Nephritis	14.64	14.40	14.53	14.52	4.442	16.3	3.19	96.0	1.08	39.7	1.04	1.04
71985 Toxic Goiter	15.00	13.88	14.00	14.29	5.170	13.9	2.76	94.2	0.91	41.0	0.92	0.99
71960 Cholecystitis and Cholelithiasis	14.13	13.72	13.84	13.90	4.960	14.0	2.80	91.8	0.93	43.2	1.01	0.92
71881 Lobar Pneumonia	13.85	13.01	12.93	13.26	4.270	15.6	3.11	87.5	1.02	38.6	1.05	0.97
71812 Hypertension, Myocarditis, Asthma	13.30	13.10	13.18	13.19	4.950	13.2	2.67	87.2	0.88	41.3	0.97	0.91
72803 Cholecystitis and Cholelithiasis	13.50	12.60	13.10	13.07	4.180	15.6	3.13	86.3	1.04	40.7	1.13	0.91
72029 Toxic Goiter	12.81	12.63	12.73	12.72	4.600	13.8	2.74	84.0	0.92	34.0	0.86	1.06
71841 Cholecystitis with Cholelithiasis. Achlorhydria	12.59	12.44	12.70	12.58	4.110	15.3	3.06	82.9	1.01	36.9	1.04	0.96
71956 Migraine	12.92	12.23	12.08	12.41	4.600	13.5	2.70	82.0	0.89	38.6	0.98	0.91
72478 Pelvic Peritonitis	12.30	12.13	12.13	12.19	4.890	12.5	2.49	80.5	0.92	38.2	0.91	0.91
68797 Pernicious Anemia	12.20	12.30	11.75	12.08	3.290	19.4	3.67	79.7	1.25			
72053 Bronchiectasis	12.32	11.48	11.66	11.82	4.220	14.0	2.80	78.2	0.93	35.6	0.98	0.94
72032 Dietary Deficiency, Protein Deprivation, Achlorhydria	12.15	11.64	11.47	11.75	3.480	16.9	3.38	77.6	1.11	34.1	1.13	0.98

TABLE III—CONT'D

DESIGNATION OR DIAGNOSIS	HEMOGLOBIN GRAMS PER 100 C.C. BLOOD				ERYTHROCYTE COUNT, MILLIONS PER C.MM.	HEMOGLOBIN COEFFICIENT	PIGMENT/CELL RATIO	HEMOGLOBIN PER CENT	COLOR INDEX	CELL VOLUME PER CENT	VOLUME INDEX	SATURATION INDEX
	OXYGEN CAPACITY	PROTEIN IRON	ACID HEMATIN	THREE-METHOD AVERAGE								
71989 Syphilis, Glaucoma	11.88	11.64	11.70	11.74	3.960	14.9	2.96	77.5	0.98			
72218 Pernicious Anemia. Posterolateral Sclerosis	10.34	10.38	10.57	10.43	2.676	19.6	3.90	69.0	1.29	32.0	1.38	0.93
72522 Bacterial Endocarditis	10.40	10.28	10.47	10.38	3.540	14.7	2.93	68.6	0.97	32.4	1.06	0.92
72577 Acute Rheumatic Fever	10.20	9.42	9.60	9.74	4.080	11.9	2.39	64.2	0.79	30.0	0.86	0.92
72119 Chronic Myelogenous Leucemia	9.70	9.52	9.42	9.55	3.090	15.4	3.09	63.0	1.02			
71816 Neurosyphilis. Therapeutic Malaria	9.68	9.09	8.99	9.25	3.400	13.6	2.72	61.0	0.90	25.0	0.85	1.05
72504 Hypotension. Addison's Disease	9.10	8.28	8.63	8.67	3.380	12.8	2.56	57.2	0.85	28.4	0.98	0.87
72109 Hypertrophy of Prostate. Pyelonephritis	7.07	7.15	7.67	7.29	2.310	15.8	3.16	48.1	1.04	21.6	1.08	0.96
71780 Pernicious Anemia	4.56	5.12	5.15	4.94	1.310	18.9	3.77	32.6	1.25			
72230 Bacterial Endocarditis	2.97	3.03	3.29	3.10	1.324	11.7	2.34	20.5	0.78	11.4	0.98	0.79

solution was made for enumeration of blood cells. The count reported represents the average of four to eight fills of the counting chamber. Bureau of Standards equipment was used. Hematocrit determinations were made in duplicate after saturating with oxygen in sedimentation tubes and centrifuging to constant values.

The oxygen capacity method used was that of Van Slyke and Neil.[7] Hemoglobin determinations based on iron were made by two separate technics recently devised in this laboratory.[1] In one hemoglobin was calculated from a colorimetric estimation of protein iron as Prussian blue, and in the other by comparing the acid hematin color with an acid hematin standard of known iron content.

The results of our observations appear in Tables I, II, and III. The hemoglobin as determined by protein iron throughout the series is found to be slightly lower than that found by oxygen capacity. In general, however, the agreement between the two methods is excellent. We have, therefore, used the average of the three methods for comparison with the hemoglobin content of blood as found by other observers. This comparison will be seen

TABLE IV

COMPARISON WITH PREVIOUSLY REPORTED OBSERVATIONS ON NORMAL INDIVIDUALS

	NUMBER OF OBSERVATIONS		MEAN ERYTHROCYTE COUNT MILLIONS PER C.MM.			HEMOGLOBIN					
						MEAN CONCENTRATION GRAMS PER 100 C.C.			COEFFICIENT		
	MEN	WOMEN	MEN	WOMEN	MEN AND WOMEN	MEN	WOMEN	MEN AND WOMEN	MEN	WOMEN	MEN AND WOMEN
Osgood and Haskins 1926-1927	153	100	5.60	4.80	5.20	16.0	13.69	14.85	14.29	14.29	14.29
Wintrobe 1929-1930	100	50	5.85	4.93	5.39	17.0	13.76	15.38	14.53	13.97	14.25
Haden 1922-1932	70	30	4.95	4.38	4.67	15.34	13.37	14.35	15.50	15.26	15.38
Total	323	180									
Averages of Three Series			5.47	4.70	5.08	16.11	13.61	14.85	14.77	14.51	14.64
Broun and Briggs 1932-1933											
Oxygen Capacity	23	13	5.29	4.78	5.03	16.76	14.15	15.45	15.84	14.80	15.46
Protein Iron	23	13	5.29	4.78	5.03	16.53	13.82	15.18	15.62	14.45	15.21
Acid Hematin Colorimetric	23	13	5.29	4.78	5.03	16.53	14.06	14.29	15.62	14.70	15.27
Three-Method Average	23	13	5.29	4.78	5.03	16.60	14.01	15.30	15.67	14.67	15.33

in Table IV. The figures of Osgood and Haskins, Wintrobe, and Haden are quoted from the article of Haden to which we have already referred. It will be noted that the figures obtained by averaging the results of these three series of observations are in more general agreement with our results than are those of any one individual series of observations. Perhaps the most important figure in the table is that termed "hemoglobin coefficient," indicating grams of hemoglobin per 100 c.c. blood when the erythrocyte count is 5,000,000 per c. mm. In regard to this figure the results of Haden are in much closer agreement with our figures than are those of Osgood and Haskins and Wintrobe.

The figure designated as "Pigment-Cell Ratio" is obtained by dividing the grams of hemoglobin per 100 c.c. blood by the millions of erythrocytes per c. mm. of blood. The figure obtained actually represents the milligrams of hemoglobin present in 100,000,000 erythrocytes. Could the precedent be established of using this figure in place of the conventional color index, it would encourage the practice of reporting hemoglobin in terms of grams per 100 c.c., and the color index, if desired, could be obtained approximately by dividing this figure by three as suggested by Cullen.[2] However, it seems obvious to us that the practice of reporting hemoglobin per cent and color index might well be abandoned for the reason that the normal hemoglobin value is much higher in men than in women. Calculations from an arbitrary standard are, therefore, necessary although undesirable. In the present study hemoglobin per cent was calculated from the mean of the average hemoglobin coefficients for normal men and women.

The average erythrocyte count for normal men was found to be 5.29 millions per c. mm. The average erythrocyte count for normal women was 4.62 millions.

The average hemoglobin value for men was 16.60 gm., with variations between 15.12 and 18.03 gm. For women the average hemoglobin value was 14.0 gm., ranging between 12.18 and 15.39 gm.

The average hemoglobin coefficient for men was 15.7 gm.; for women, it was 14.7 gm.

The average cell volume per cent for normal men was 46.57 per cent. For normal women it averaged 40.37 per cent. The mean of the averages of the two sexes was 43.47 per cent. The mean of the average of the two sexes per 5,000,000 erythrocyte count was 43.1 per cent.

The average saturation index of normal men was observed to be 1.017. That of normal women was likewise 1.017.

The pigment-cell ratio averaged 3.15 for the normal males and 2.95 for the normal females. The mean ratio was, therefore, 3.05. The normal limits for this figure were found to be between 2.44 and 3.51. It can be noted that a high figure was found in the two cases of pernicious anemia observed, namely, 3.67 and 3.90 and a low figure in the achromic anemias as shown by cases 72577 and 72230, namely, 2.39 and 2.34. Hence this figure can be used as well as the color index to determine the type of anemia.

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A COMPARISON OF METHODS FOR THE DETERMINATION OF URIC ACID IN HUMAN, BOVINE, AND AVIAN BLOODS*†

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NUMEROUS methods for the determination of uric acid in human blood have been developed, the chief motive being to incorporate the higher degree of accuracy of the isolation methods with the greater simplicity and ease of manipulation of the direct methods. While several of these methods have given satisfactory results on human blood, few of them have been applied to the blood of other animals. Since in this laboratory we have been especially interested in the analysis of bovine and avian (chicken) bloods, the present study was undertaken to ascertain the most satisfactory method for the determination of uric acid in these bloods.

DISCUSSSION OF DATA

In Table I data are presented to show the results of analyses of human, bovine, and avian bloods, using the Folin-Wu laked blood filtrate[15] and the Folin unlaked blood filtrate.[12] The methods of Folin[11, 13] of Folin as modified by Bulmer, Eagles and Hunter,[8] of Benedict,[2] and of Brown[7] were used with each of these filtrates, each method being applied to the filtrate directly and to the uric acid isolated as the silver salt from each filtrate. Thus twelve methods were used with each sample of blood.

The data in Table I show that in most cases the isolation methods give lower results than the direct methods. This fact is more evident in the case of laked than in the case of unlaked blood filtrates, the difference being especially pronounced in the case of laked filtrates from bovine blood. Direct and

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TABLE I

SHOWING A COMPARISON OF THE URIC ACID CONTENT OF HUMAN, BOVINE, AND AVIAN BLOODS BY THE METHODS OF FOLIN, BENEDICT, AND BROWN ON LAKED AND UNLAKED BLOOD FILTRATES

SPLE. NO.	LAKED BLOOD						UNLAKED BLOOD					
	FOLIN		BENEDICT		BROWN		FOLIN		BENEDICT		BROWN	
	DIRECT	ISOLATION*	DIRECT	ISOLATION	DIRECT	ISOLATION	DIRECT	ISOLATION*	DIRECT	ISOLATION	DIRECT	ISOLATION
HUMAN	MG. URIC ACID PER 100 C.C. OF BLOOD											
1	4.2	3.2	3.4	2.9	3.7	2.9	3.1	2.4	2.8	2.8	3.5	3.2
2	5.2	4.1	4.5	3.2	5.6	4.5	5.4	4.1	4.8	3.8	5.9	4.6
3	4.6	3.3	3.6	3.0	3.5	3.7	3.4	3.5	2.7	2.6	5.2	4.1
4	5.7	4.9	4.8	4.5	4.5	3.9	4.6	2.8	4.6	5.3	5.3	3.0
5	5.3	4.6	4.2	3.6	5.4	4.7	4.4	4.6	4.1	4.7	5.8	5.8
6	3.9	2.8	3.1	3.0	4.6	3.6	3.3	3.2	3.2	3.2	4.3	4.2
7	4.3	3.4	3.6	3.1	4.6	3.7	3.4	3.4	2.9	3.4	3.8	4.0
Average	4.74	3.76	3.89	3.32	4.56	3.86	3.94	3.42	3.58	3.69	4.83	4.13
BOVINE												
1	2.2	1.5	1.4	0.7	2.0	no color	0.5	1.3	1.4	0.3	1.2	no color
2	2.5	0.7	2.6	0.6	1.4	0.4	0.6	0.3	0.7	0.4	0.9	no color
3	2.5	0.6	2.2	0.8	1.4	0.9	0.7	0.4	0.7	0.7	0.6	no color
4	2.7	0.7	2.0	0.6	1.8	0.6	0.5	0.5	0.8	0.5	0.6	no color
5	2.2	0.7	2.0	0.6	1.7	0.7	0.8	0.5	0.9	0.5	0.6	no color
6	1.9	0.9	1.9	0.6	1.7	0.7	0.5	0.4	0.7	0.6	0.6	no color
Average	2.33	0.85	2.01	0.65	1.67	0.66	0.60	0.57	0.87	0.50	0.75	no color
AVIAN												
1	4.6	3.0	4.1	2.8	4.4	lost	4.1	3.2	3.8	3.0	4.3	lost
2	4.6	3.5	4.4	3.3	5.8	4.1	5.0	3.5	4.8	3.6	5.3	4.6
3	5.2	3.4	4.8	3.7	5.5	3.4	4.5	4.1	5.1	4.1	5.9	3.9
4	3.9	2.1	3.7	2.0	4.6	2.6	3.0	2.6	3.1	2.9	3.5	2.7
5	4.1	3.5	4.2	3.2	4.8	3.5	3.9	3.1	3.9	lost	5.2	4.9
6	3.9	2.3	3.9	2.3	4.6	2.8	3.1	2.2	3.1	2.2	4.8	3.2
7	5.1	5.5	5.4	4.9	8.1	7.2	7.0	6.0	5.4	5.6	8.0	7.5
8	3.5	3.3	4.5	3.6	6.1	5.0	3.4	4.1	4.3	3.8	5.8	4.0
9	3.1	2.6	3.9	2.6	4.3	2.8	3.0	2.6	4.2	3.4	4.7	3.3
10	2.6	1.8	2.8	1.7	4.1	1.5	2.0	1.6	2.6	1.9	3.2	2.1
11	4.3	4.0	5.1	3.9	6.1	4.0	4.3	3.0	4.9	3.7	5.1	4.5
Average	4.08	3.18	4.25	3.09	5.31	3.69	3.93	3.27	4.11	3.42	5 07	4.07

All figures are averages of duplicate determinations.

*Five cubic centimeters of silver lactate solution were used in these cases as suggested by Bulmer, Eagles, and Hunter.[8]

isolation procedures agree more closely on unlaked than on laked filtrates. All isolation procedures give results which agree fairly well.

With regard to the great differences in uric acid values obtained by the direct and isolation procedures on bovine blood, the work of Benedict and his coworkers[9, 10, 17] should be mentioned. They have isolated from the erythrocytes of bovine blood a combined form of uric acid which is not precipitated by silver-magnesia mixture. The low values obtained with the isolation method and with the direct methods on unlaked filtrates from bovine blood might be explained on this basis, since combined uric acid would not be precipitated by the silver lactate in the isolation procedure and, being in the corpuscles, would not be present in unlaked blood filtrates.

Bulmer, Eagles, and Hunter[8, 16] ascribed the discrepancy between the direct and isolation methods to a substance "X" which they isolated from animal bloods, including bovine blood, and which they called a simple pyrimidine nucleoside. This compound is precipitated by silver lactate but is not liberated by acid sodium chloride, so that it would not appear in the isolation procedure.

Almost simultaneously, Benedict and his coworkers[1, 3, 4] isolated from pig and human bloods an interfering substance "thiasine" or thioneine, as it was later called. They claimed that this compound is not the same as substance "X," and that it causes high values in the direct method.

From the averages given in Table I it appears that for human blood Benedict's direct method applied to Folin's unlaked blood filtrate gives results more closely agreeing with the isolation procedure than any of the other methods. In the case of bovine blood, Folin's direct method applied to an unlaked blood filtrate gives results agreeing most closely with the isolation procedure. In the case of avian blood, the agreement between the direct and isolation procedures on an unlaked filtrate is about the same for Folin's as for Benedict's method.

Brown's method, with one exception, gives higher results on human and avian bloods than the other methods. In the case of bovine blood, there was no color produced when Brown's isolation procedure was applied to the unlaked filtrate. When Brown's direct method was applied to a laked blood filtrate, the results were lower than the direct methods of Folin and of Benedict.

In 1931 Benedict[5] suggested the use of tungstomolybdic acid for the preparation of laked blood fitrates. He[6] also suggested a modification of the isolation procedure. In Table II Benedict's laked blood filtrate is compared with Folin's laked and unlaked filtrates from human, bovine, and avian bloods. Folin's direct[13] and isolation[8] methods are applied to all three filtrates, and Benedict's direct[2] and new isolation[6] procedures are used on Benedict's laked filtrates.

From the averages in Table II it appears that in the case of human blood results by all the isolation methods agree closely. Of the direct methods that of Folin, used with his unlaked filtrate, gives results which agree most closely with the results obtained by the isolation procedure. It should be noted, however, that in Table I, with an unlaked filtrate, results by Benedict's direct method agreed more closely with the isolation procedure than did Folin's.

TABLE II

SHOWING A COMPARISON OF THE URIC ACID CONTENT OF HUMAN, BOVINE, AND AVIAN BLOODS BY THE DIRECT AND ISOLATION METHODS OF FOLIN AND BENEDICT ON UNLAKED AND TWO TYPES OF LAKED BLOOD FILTRATES

SPLE. NO.	LAKED-FOLIN		LAKED-BLOOD-BENEDICT				UNLAKED-FOLIN	
	FOLIN		BENEDICT		FOLIN		FOLIN	
	DIRECT	ISOLATION*	DIRECT	NEW	DIRECT	ISOLATION*	DIRECT	ISOLATION*
HUMAN	MG. URIC ACID PER 100 C.C. OF BLOOD							
11	3.0	2.7	3.3	2.4			3.0	2.5
12	3.2	2.5	3.1	2.5			2.5	2.7
13	2.8	2.8	2.9	2.0			2.5	2.4
14	3.9	3.2	2.7	3.5			3.1	2.9
15	2.8	2.2	2.8	2.3	2.9	2.3	2.9	2.1
16	3.6	3.5	3.3	3.2	3.7	3.5	3 6	3.5
17	2.8	2.5	3.4	2.4	3.5	2.5	2.4	2.1
Average	3.16	2.77	3.07	2.61	3.36	2.76	2.86	2.60
BOVINE								
11	1.6	0.4	1.6	0.9	1.6	0.5	0.5	0.2
12	1.3	0.4	1.6	0.9	1.5	0.5	0.4	0.2
13	1.0	0.5	1.2	0.5	1.3	0.6	0.4	0.2
14	1.9	0.7	2.0	1.0	1.8	0.7	0.6	0.5
15	1.4	0.5	1.6	0.8	1.5	0.5	0.4	0.3
16	1.5	0.6	1.6	1.0	1.7	0.7	0.5	0.4
Average	1.45	0.51	1.60	0.85	1.57	0.58	0.47	0.30
AVIAN								
11	2.9	1.8	4.2	2.9	3.2	1.8	2.0	1.7
12	2.9	2.1	3.8	3.0	3.2	1.9	2.4	2.1
13	3.3	2.3	4.9	2.7	3.7	2.3	2.5	2.3
14	3.3	2.3	4.4	2.7	3.4	2.3	2.8	2.3
15	3.7	2.7	5.3	2.9	4.0	2.9	2.8	2.9
16	3.5	2.5	4.6	2.7	3.7	2.6	2.6	2.3
Average	3.27	2.28	4.55	2.81	3.53	2.30	2.51	2.27

All figures are averages of duplicate determinations.

*Five cubic centimeters of silver lactate solution were used in these cases as suggested by Bulmer, Eagles, and Hunter.[8]

With bovine blood results by the isolation method do not agree so closely as they do with human blood. Benedict's new method gives the highest values, while Folin's method, applied to his unlaked fitrate, gives the lowest values. In Table I Benedict's isolation procedure applied to Folin's unlaked blood filtrate gives lower results than Folin's isolation procedure.

In the case of avian blood, Benedict's new method gives higher results than any of the other procedures. Folin's method with his unlaked filtrate gives the closest agreement between direct and isolation procedures although in Table I the agreement is nearly as close when Benedict's procedure is used with Folin's unlaked filtrate.

In conclusion, it may be stated that for human and avian bloods, it makes little difference which method is used, provided an isolation procedure is followed. Of the direct methods those of Benedict and of Folin, with Folin's unlaked filtrate, give results agreeing closely with the isolation procedures. For bovine blood Folin's direct method, applied to his unlaked filtrate, agrees most closely with the isolation procedure. The authors have found Folin's method more satisfactory than Benedict's because the use of the urea-cyanide solution eliminates the formation of a troublesome precipitate which is always imminent when Benedict's method is used.

Since this paper was written, Folin[14] has made several revisions in his direct method and in the preparation of his uric acid reagent. While lack of time prevents the repetition of the work here presented, it is felt that Folin's latest technic should be considered in selecting a method for the determination of uric acid in animal bloods.

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BLOOD IODINE STUDIES*

III. A Simple Reservoir Buret for Making Microtitrations

Francis J. Phillips, and George M. Curtis, Columbus, Ohio

THE quantitative determination of the minute amount of iodine normally present in the blood and urine requires special precision apparatus. In developing an adequate method it became necessary for Davis[1] to construct a suitable microburet for making the titrations. This was a modification of the original reservoir buret designed by Professor F. C. Koch of the Department of Physiological Chemistry of the University of Chicago. Koch's buret was

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adequate for making the usual microtitrations. It was a decided improvement over the old style of buret in common use.

The titration of the minute amount of iodine present in 10 c.c. of blood, however, required even a more precise buret. It became necessary to employ one accurately graduated to thousandths of a cubic centimeter. We are describing and figuring the simple reservoir buret, Fig. 1, which has finally been developed as a result of this need. This has given satisfaction in the routine daily determinations of the minute amounts of iodine normally present in the blood and urine.

A Kahn 0.2 c.c. graduated pipette was fused to the open end of the tube on the one-stem side of a three-way microbore stopcock. On the two-stem

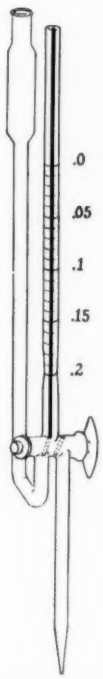

Fig. 1.

side of the three-way stopcock, one tube is drawn to a fine capillary bore and cut 18 cm. from the stopcock, Fig. 1. This length is necessary for titration convenience. The other tube on the two-stem side of the stopcock is bent in a "U" shape. The open end is drawn parallel to the 0.2 c.c. pipette, and about 2 cm. behind it. One centimeter above the level of the zero mark on the 0.2 c.c. pipette a 10 c.c. cylinder-shaped reservoir is fused to the open end of the "U" tube. The accompanying figure of the buret used in this laboratory should be adequate as a design for any competent glass blower to follow in making this apparatus.

The buret described above has been in use in the iodine laboratory for several months. The titrations in the routine daily work here require the measurement of 0.002 c.c. increments of a 0.001 normal sodium thiosulphate

solution. With the above described buret these measurements have been accurately and conveniently carried out.

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1. Davis, C. B., and Curtis, G. M.: The Quantitative Determination of the Iodine Content of Blood, J. LAB. & CLIN. MED. 18: 24, 1932.

CAGE FOR MICE AND RATS*

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THIS type of cage has been used by us for the past two years, and we feel that it possesses distinct advantages over other types of cages used heretofore. In our experience some of these advantages consist in the prevention of food and bedding pollution by the segregation of each in a different compartment, the ease and cheapness of construction, the greater accessibility of the animals, the ease of cleaning, the ease of observation of the undisturbed animal and the ease of transportation from place to place. The cage serves well for feeding experiments, breeding purposes, stock animals, and general experimental purposes. It is compact, economical of space, and may be placed on a narrow shelf. Waste cotton is employed for bedding and it is placed in the upper compartment. If so desired, the food may be placed in the upper rather than the lower compartment.

Dimensions.—11.25 x 8.5 inches.

Materials.—Galvanized hardware cloth (¼ inch mesh) for body, door, removable floor, and as a guard around opening between the two compartments (to prevent bedding material from falling through into lower compartment); sheet zinc (1/32 inch) for floor of upper compartment, card holder, and to form margin of door opening; sheet metal (1/16 inch) for band at bottom of cage and door catch; heavy wire soldered to edge of mesh door (to provide additional strength and support); water container (6 inch test tube, single hole rubber stopper, glass tubing) fastened by detachable clips to side of body; enamel pan.

*From the School of Medicine, University of Alabama.
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MAINTAINING WATER AND AIR BALANCE DURING PROLONGED INCUBATION*

Experiences Gained from Growing Tubercle Bacilli.

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DURING the course of years of experience, one frequently stops to glance back and smile at the arbitrariness of formed habits and empiricism which proved exceedingly wasteful of time and material and which condition could easily have been remedied by opportunity for a few moments of proper experimentation and test. Of course, the apology might be offered that the majority

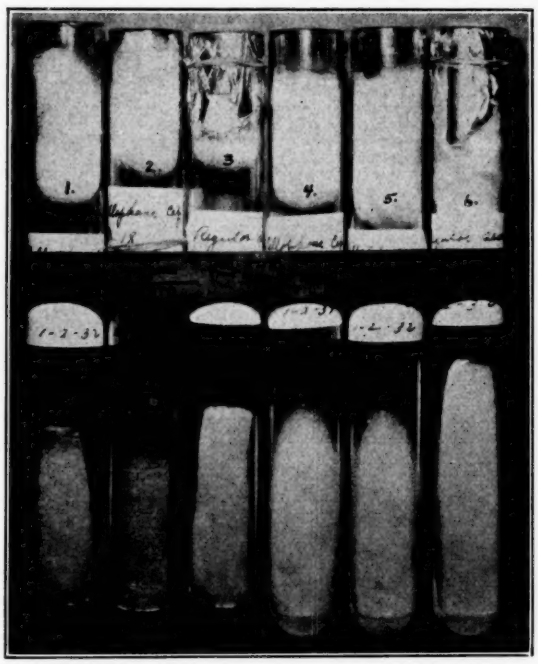

Fig. 1.—The result of capping with cellophane or tin foil one week after incubation at 37° C. upon potato cylinder and inspissated egg yolk medium. Tubes 1 to 3 are the potato medium and Tubes 4 to 6 the egg medium. Tubes 1 and 4 were capped with cellophane over a cotton plug, the cellophane cap over Tubes 2 and 5 was perforated with pin holes and Tubes 3 and 6 were capped with tin foil over a paraffined cotton plug. Note the drying of the top of the medium in the cellophane capped Tubes 1, 2, 4, and 5.

of us have been trained to follow instructions implicitly and not question our textbooks or teachers. In growing tubercle bacilli the technical phases are strikingly different, and it was a stroke of "genius" that led Koch to discover the tubercle bacillus, or rather let us say correctly, in place of genius, his patience and perseverance. It is now fifty years since this discovery was made

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and bacteriologists in general have failed to recognize fully the technical obstacles encountered in growing tubercle bacilli. Aside from the composition of the medium which has been elaborately studied by us,[1, 2, 3] there are two factors dependent upon technical detail that may spell success or failure in growing these bacilli, the maintenance of the water and air balance. To attempt to maintain the water balance of the medium by any means of supplying water in the incubator or in the cans containing the culture tubes or flasks is obviously futile as only experience will prove, or possibly a little reflection upon mother's bread box, or the storekeeper's unceasing vigil to

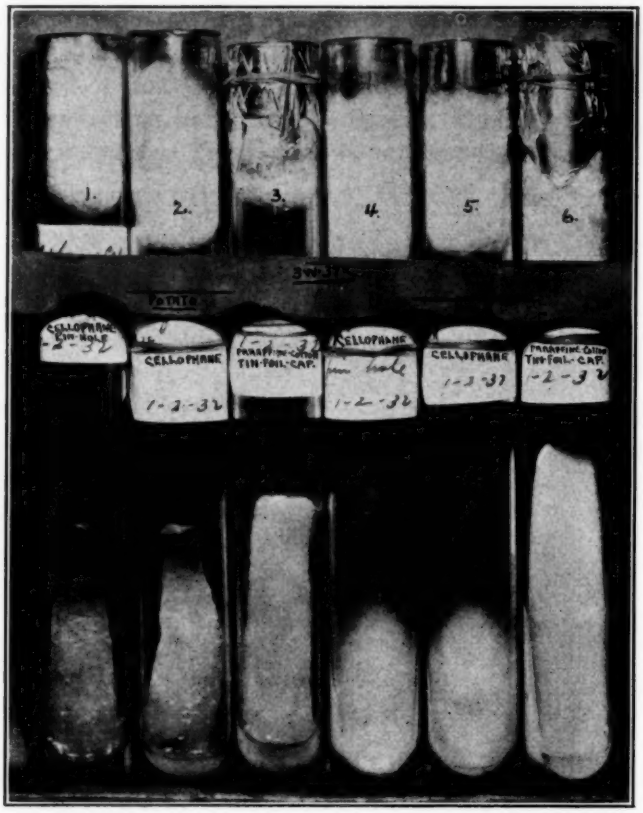

Fig. 2.—The same tubes shown in Fig. 1, after three weeks at 37° C. Note the marked drying of the mediums in Tubes 1, 2, 4, and 5 (cellophane capped) and the evaporation of the condensation water in the bottom of the tubes.

prevent mold disasters, will provide the evidence. And molds extend into the smallest crevices, even through the bacteriologic impervious cotton plug if given time and moisture. Dryness is the enemy of mold and, therefore, it is better to maintain water balance by preventing evaporation; and yet one may ask how to maintain air balance and still prevent evaporation in a dry incubator, particularly in a dry climate of extremely low humidity and over a long period of time. The investigator soon learns that explanations must await facts, and so the experiences to be recorded will be presented as facts which anyone can easily explain on the basis of present knowledge.

It has been the custom of the senior author over many years in growing tubercle bacilli to utilize the method of impregnating cotton plugs or culture tubes with hot paraffin, and then covering the tubes with tin foil or cloth impregnated with paraffin, with the cap secured by a small rubber band to prevent drying of the medium. In practice this method had met the needs apparently satisfactorily, when a trial of cellophane capping was advocated to us as being more easily used and readily applied to the tubes or flasks. The objection raised by us to the use of cellophane was based on our knowledge of the water and moisture permeability of this material, and especially

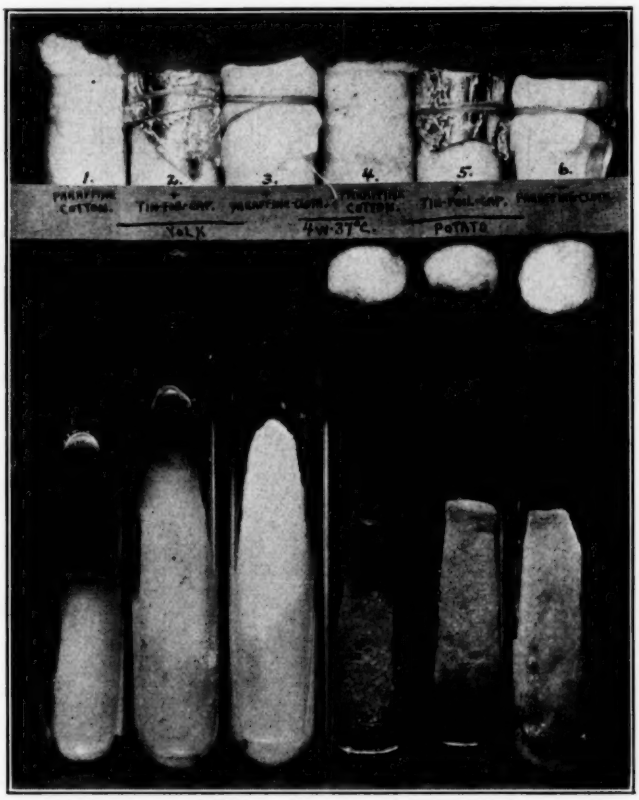

Fig. 3.—A comparison of capping with tin foil or paraffined cloth. Tubes 1, 2, and 3 are inspissated egg yolk medium, Tubes 4, 5, and 6 potato cylinder medium. Tubes 1 and 4 are controls plugged with paraffined cotton. Tubes 2 and 5 are in addition capped with tin foil and Tubes 3 and 6 are capped with paraffined cloth. Note the greater drying of the medium and condensation water in Tubes 1 and 4 uncapped, and the better maintenance of water balance in the paraffin cloth capped Tubes 3 and 6 as compared to the tin foil capped Tubes 2 and 5 after four weeks at 37° C.

because one of us has had intimate knowledge of its use for perevaporation. Those advocating the use of cellophane capping maintained it would save labor and obviate the use of the rubber band as well as paraffining of the cotton plug, and, of course, would make easier the access of air (an erroneous deduction). It was tried with the results shown in Figs. 1 and 2. In commenting on this test it may be noted that very little difference in the rate of drying of the medium occurred regardless of whether the cellophane had been perforated or not.

There are a number of methods which have been variously advocated for maintaining the water balance in culture tubes over relatively long periods of time of incubation, and two of them were cited above, the tin foil capping or paraffin cloth capping, but we are not aware of actual tests recorded in the literature of the relative merits of these two. To us they appeared equally efficient and the tin foil, although more costly, possessed the advantage of being less messy and less bulky. Test, however, proved the paraffin cloth to be more efficient as shown in Fig. 3. In some laboratories it is the

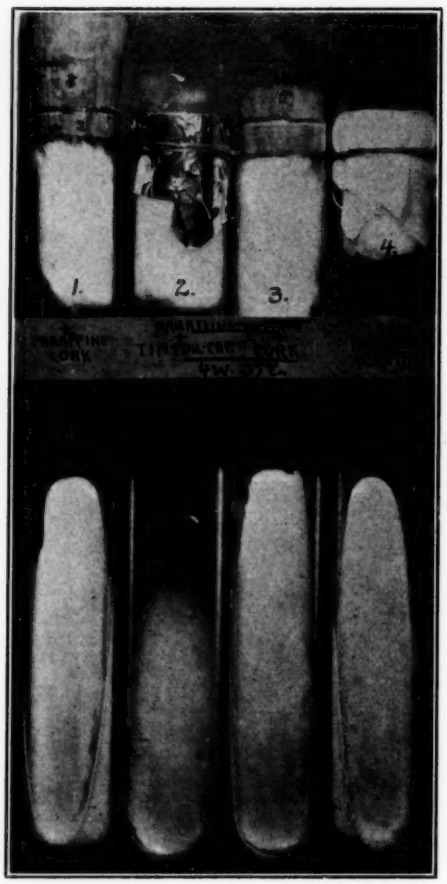

Fig. 4.—A comparison of capping with cork stoppers and tin foil or paraffin cloth after keeping inspissated egg yolk medium for four weeks at 37° C. Note the absence of drying in the tubes stoppered with the plain nonparaffined cork (Tube 3) and with the paraffined cork (Tube 1) as well as with the paraffin cloth cap while Tube 2 tin foil capped showed evidences of drying of the upper portion of this medium at this time.

custom to merely pour a layer of hot paraffin over the cotton plug without attempting to impregnate all the fibers, and then to perforate the paraffin plug with a few pin holes. This method is fairly satisfactory but fails to remove entirely the water absorbing powers of the cotton plug, and each time that the tube is to be opened the plug of paraffin must be removed, which in our hands has proved less satisfactory than other methods, including the paraffin cloth which with the paraffin impregnated cotton plug is more efficient for preventing water evaporation.

Recently one of us[4] described a tissue substrate method for practice, and for simplicity recommended the use of a lightly paraffined cork. It seemed advisable, therefore, to determine the merit of the cork for the purpose of maintaining water and air balance in culture tubes. The maintenance of water balance by the cork placed over the paraffin impregnated cotton plug is well illustrated in Fig. 4.

However, this experiment proved only the adequacy of the cork for maintaining water balance, and our knowledge regarding the use of the cork

Fig. 5.—The results of capping with tin foil, with the cork stopper (not paraffined) and a paraffined cork stopper upon the growth of small numbers of tubercle bacilli (a human strain) on an inspissated egg yolk medium after three months' incubation at 37° C. All the cotton plugs were impregnated with hot paraffin, and Tubes 1 and 2 were capped with tin foil, Tubes 3 and 4 were stoppered with a plain dry cork, and Tubes 5 and 6 with a paraffined cork inserted fairly tightly. Note that the growth of the bacilli in the plain corked Tubes (3 and 4) is equal to that in the tin foil capped tubes (1 and 2) in both the 0.000,1 mg. plantings (Tubes 1 and 3) and in the 0.000,001 mg. plantings (Tubes 2 and 4), while in both plantings the growth was retarded in the tubes stoppered with the paraffined corks (Tubes 5 and 6). The latter finding is readily understood on the basis of the relative imperviousness of the paraffined cork to air especially when tightly inserted into the tube.

as a stopper for effervescing and fermenting beverages made us doubt its value in maintaining air balance. Manometric tests assured us that the dry cork was readily pervious to air, but that the cork heavily impregnated with paraffin and tightly inserted into a tube was almost completely impervious to air and was, therefore, unsuited to our use for growing tubercle bacilli.

If the cork was only lightly impregnated with paraffin and was only loosely inserted into the tube, it would not prevent air access in sufficient amount for growing tubercle bacilli. It is well also to remember that the water wet cork is far less pervious to air than the dry cork. To further test the value of the dry cork, it was given a thorough trial in culture tests with tubercle bacilli in graded seedings (see Fig. 5), and proved sufficiently valuable both from an economic and efficiency standpoint so that it is now used in all our culture tube work in place of tin foil or paraffin cloth, being inserted above the cotton plug which has been impregnated completely but lightly with hot paraffin. The corks can be repeatedly used and can be freed from paraffin, if desired, by washing in gasoline or some other readily volatile paraffin solvent, after which they may be air dried and, finally, to free from traces of the solvent, may be placed in the dry sterilizer for an hour or two.

The efficiency of the paraffining procedure in maintaining water and air balance in culture flasks is well illustrated by a report just presented by us[5] in which we found that tubercle bacilli remained viable for over twelve years after growing in nursing bottles containing broth, stoppered with a paraffined cotton plug, and covered with paraffined cloth. Some of these bottles lost as little as one-quarter to one-half ounce of liquid out of an original three ounces placed in the flask twelve years previously.

In conclusion, it may be stated that the problem of maintaining water and air balance in culture tubes and culture receptacles appears to be one of preventing the conduction of moisture from these receptacles by avoiding the use of moisture absorbent materials, or by satisfactorily impregnating these with a completely nonabsorbent and water impervious material, such as paraffin. It also appears that this can be attained without appreciable influence upon adequate gaseous exchange, especially sufficient to maintain conditions required for the multiplication of slow growing microorganisms, such as the tubercle bacillus.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LIVER, Silver Nitrate Reaction and Its Applicability to the Diagnosis of Diseases of, Rosa, L. Wien. Klin. Wchnschr. **46**: 1104, 1933.

Rosa describes the test below as of value in the diagnosis of hepatic diseases.

Set up 5 test tubes and introduce the following amounts of urine: 1, 2, 1.5, 2, and 5 c.c. Then add the following amounts of 10 per cent solution of silver nitrate: 0.2, 0.2, 0.1, 0.1, and 0.1 c.c. The resultant dilutions are thus 1:5, 1:10, 1:15, 1:20, and 1:50. While frequently shaking boil the tubes for from thirty to forty seconds in a water-bath. Remove from the bath and allow the precipitate to settle.

Negative reaction: sediment, snow white; supernatant fluid transparent yellow.

Positive reaction: lilac, brown, or black precipitate with supernatant fluid of same color.

The more severe the pathologic process, the higher the dilution in which the reaction occurs.

The reaction is related to the sodium chloride concentration of the urine.

TUBERCULOSIS, Vernes Flocculation Test in, Murray, R. South African M. J. **7**: 645, 1933.

From a study of 121 cases Murray concludes that the sedimentation rate and the Vernes test do not appear to be related in any simple manner.

In definitely diagnosed cases of tuberculosis the Vernes test would not appear to be invariably positive, there being negative results in some 6.4 per cent of cases.

In cases where recovery is taking place, the sedimentation test appears to give a normal reading sooner than the Vernes test.

MILK, Preservation of for Streptococcus and Abortus Examination, Bryan, C. S. Am. J. Pub. Health **23**: 1182, 1933.

A 1:10,000 concentration of brilliant green (0.1 c.c. of 1 per cent aqueous solution to 10 c.c. of milk) was found highly satisfactory.

MALARIA, Vital Staining of Mitochondria in, Boucher, H. Compt. rend. Soc. de biol. **112**: 882, 1933.

Mitochondria can be demonstrated in the malaria parasite *Plasmodium vivax* by vital staining with Janus green.

The stain is prepared as follows: Of a saturated solution in absolute alcohol of neutral red 0.4 c.c. is added to 10 c.c. of absolute alcohol to which is added also three drops of a saturated alcoholic solution of Janus green. A drop of this is allowed to dry on a perfectly clean slide and the moist blood preparation is made on the thin film of stain. The mitochondria in the form of spherical granules stained by the Janus green half a micron in diameter vary in number from 1 to 20 according to the size of the parasite. The neutral red stains granules in the red blood corpuscles, the rose coloration assumed by the cytoplasm of the parasite being due to a reduction product of the Janus green.

BLOOD CHOLESTEROL, In Chronic Nephritis and Retention Uremia, Ashe, B. I., and Bruger, M. Am. J. M. Sc. **186:** 670, 1933.

In cases of chronic Bright's disease with marked nitrogen retention, a low plasma cholesterol foretells death in uremia. In most cases this low reading is observed at an appreciable interval before death; in rare cases such a level is found only about twenty-four to forty-eight hours before exitus.

An elevated plasma cholesterol indicates a good prognosis as to recovery from preuremic symptoms but does not preclude the possibility of death from causes other than uremia.

There appears to be no close relation between the height of the plasma cholesterol and the degree of edema in patients with chronic Bright's disease.

There is some clinical evidence of a reciprocal relation between the blood urea and the plasma cholesterol; at other times this relation is offset by other factors. A constant inverse relation between the plasma proteins and cholesterol was not observed.

The diminished cholesterol content of the plasma observed in cases of Bright's disease dying of retention uremia is probably due to such contributing factors as cachexia and anemia.

FIBRINOGEN, Quantitative Determination of in the Blood, Boehm, G. Diagn. E. Techn. di Lab. **4:** 683, 1933.

The author describes a simple technic as follows:

1. Fill and empty a sterile 10 c.c. glass syringe with 20 per cent potassium oxalate, thus leaving a thin film in the syringe.

2. Withdraw from 6 to 7 c.c. of blood by venipuncture, place in a test tube and centrifuge.

3. Remove 1 c.c. of plasma and dilute with 9 c.c. of 0.85 per cent saline and from this 1:10 dilution prepare a 1:100 dilution.

4. To a series of 16 tubes from 8 to 10 mm. in diameter add plasma and saline as shown in the table below:

TUBE	PLASMA C.C.	SALINE C.C.	CONCENTRATION OF PLASMA
1	1.0 of 1: 10	0	1.0
2	0.7 of 1: 10	0.3	0.07
3	0.5 of 1: 10	0.5	0.05
4	0.4 of 1: 10	0.6	0.04
5	0.35 of 1: 10	0.65	0.035
6	0.3 of 1: 10	0.7	0.03
7	0.25 of 1: 10	0.75	0.025
8	0.2 of 1: 10	0.8	0.2
9	0.15 of 1: 10	0.85	0.015
10	1.0 of 1:100	0	0.01
11	0.9 of 1:100	0.1	0.009
12	0.8 of 1:100	0.2	0.008
13	0.7 of 1:100	0.3	0.007
14	0.6 of 1:100	0.4	0.006
15	0.55 of 1:100	0.45	0.0055
16	0.5 of 1:100	0.5	0.005

5. To each tube add 1 drop of 10 per cent calcium chloride solution and allow to stand in the ice box for thirty minutes.

6. Read for (a) the dilution showing a definite turbidity and (b) the dilution showing complete coagulation when the tube is inclined.

The amount of fibrinogen is determined by the formula:

(Unit) of fibrinogen (U.Fg.) $= \frac{1}{P}$ where p equals the amount of plasma in the tube (as shown in the table).

The author gives the normal as 40 or 60 U.Fg. per 1 c.c. of plasma.

SYPHILIS, The Value of the Sachs-Witebsky Reaction (Citochol) for the Diagnosis of, Sebastion, F. Diagn. E. Techn. di Lab. 4: 641, 1933.

The author reports favorably upon this test which he believes to be specific, sensitive, and easy to read.

His technic follows: To 0.2 c.c. of serum inactivated at 55° C. for thirty minutes add 0.1 c.c. of 1:3 Citochol-saline mixture which has been allowed to ripen for thirty minutes at room temperature.

After twenty minutes add 1 c.c. of 0.85 per cent saline solution and shake. Readings are made after one hour, positive reactions being indicated by flocculation, the mixture remaining clear and limpid in a negative reaction.

ANEMIA, Production of Erythrocytes, Reticulocytes, and Hemoglobin in, Murphy, W. P. Arch. Int. Med. 52: 829, 1933.

It is well known that the magnitude, and to some extent the rate, of the response of the reticulocytes to therapy is in part dependent on the method of staining and technic of counting the reticulocytes, the number of erythrocytes present when treatment is started and the state of health of the patients in certain respects other than the anemia.

If the various secondary factors which influence the rise in reticulocytes are assumed to be constant, the magnitude of the response of the reticulocytes will indicate the potency of the material used if it is administered in submaximal amounts. The data presented, particularly for patients treated with maximal amounts of potent material, as with a concentrated solution of liver extract administered intramuscularly, indicate that a reticulocyte response of equal magnitude may be observed following the use of varying amounts of equally potent material, that the response will occur more quickly following the initial administration of large amounts, that the magnitude of the increase in erythrocytes may not vary directly with the magnitude and duration of the rise in reticulocytes and that the rate of increase of the erythrocytes is a more accurate indication of the effectiveness and potency of substances used in the treatment of patients with pernicious anemia than is the rate and magnitude of the reticulocyte response.

The explanation for the lack of correlation between increases in reticulocytes and erythrocytes as observed following treatment with various substances is not obvious and is probably not entirely controlled by the condition of the blood-forming organs at the time treatment is started. The variation in response to treatment by different means may be dependent on the rate of absorption or the utilization of the active principle under the various methods of its administration. If this is the case, it is probable that when administered in large amounts, as by intramuscular injection, the active material presented ready for the immediate use of the blood-forming organs causes more rapid maturation of the erythrocytes, so that the proportion of mature to immature cells (reticulocytes) in the circulation is greater than when less of the potent substance is available.

Is it possible, however, that two factors are present in the various materials used in the treatment for pernicious anemia, a stimulating (or reticulocyte-producing) and a maturing (or erythrocyte-producing) one? These may occur in varying proportions in different materials which have been used, the maturing occurring in greater proportion in the more refined and concentrated liver extract for intramuscular use.

In the treatment of the patient whose blood is in a state of relapse, it is desirable that improvement of this condition be promoted as rapidly as possible. Observations indicating an unusually rapid increase in erythrocytes following the intramuscular injection of initial large amounts of a concentrated solution of liver extract (12 c.c. prepared from 400 gm. of liver) and followed by infrequent smaller injections are presented. That this material is of a high degree of potency and that treatment by this means insures optimal efficacy are well illustrated by the improvement shown.

The administration of large doses of iron during treatment with liver extract parenterally is strongly recommended. By this means one may be more certain to obtain the maximum

effect of the liver material by preventing the development of deficiency in iron as a result of the rapid maturation of erythrocytes produced by the parenteral method of therapy.

RHEUMATIC FEVER, Intravenous Vaccination With Streptococci, Wilson, M. G., Josephi, M. G., and Long, D. M. Am. J. Dis. Child. **46**: 1330, 1933.

The curves of the seasonal incidence of recurrence of rheumatic fever for subjects receiving hemolytic streptococcus vaccine therapy in 1928 and 1929 show a definite upward trend in 1931 as compared with the control group.

Twenty-three (19 per cent) of the treated subjects as compared with 15 (17 per cent) of the controls remained free from evidence of rheumatic activity for the three-year period of observation.

The annual incidence of recurrence for subjects receiving hemolytic streptococcus vaccine therapy was 48 per cent for 1929, 39 per cent for 1930 and 37 per cent for 1931.

The subjects who received a second course of treatment with hemolytic streptococcus vaccine in 1929 exhibited more recurrences in 1930 than subjects receiving only a single course of treatment.

The first series of 26 children who received typhoid vaccine intravenously in the fall of 1930 shows a diminished incidence of recurrence comparable to that observed following hemolytic streptococcus vaccine in the previous investigation.

The incidence of recurrence following streptococcus vaccine (polyvalent streptococcus B E and *Streptococcus viridans,* autogenous, individual and pooled) did not seem to be influenced by treatment.

There was no significant difference in the incidence of recurrence or manifestations of activity following intravenous therapy with various antigens.

The results secured in this investigation would seem to support the view that the temporary diminished incidence of recurrence observed to follow intravenous treatment with hemolytic streptococcus vaccine in the previous investigation was probably unrelated to therapy.

Intravenous vaccination with various antigens in the dosage used did not seem to influence the incidence of recurrence of rheumatic activity in the children studied.

THYROID DYSFUNCTION, Gastric Acidity in, Wilkinson, S. A. J. A. M. A. **101**: 2097, 1933.

Of 100 cases of hyperthyroidism, 36 per cent showed achlorhydria, and the average acid of all cases was reduced to slightly more than half the normal.

The observation is made that the incidence of achlorhydria in hyperthyroidism rises in proportion to the duration of the toxicity rather than the degree of the toxicity.

After thyroidectomy, only 10.5 per cent show achlorhydria. The average free acid is raised to about the normal value for the entire series.

A theory is offered that the depression of gastric acidity is a phenomenon of extreme sympathetic overstimulation.

Hypothyroidism, in this series at least, produces a definite tendency to hyperacidity.

ANEMIA, Chronic Idiopathic Hypochromic, Mettier, S. R., Kellogg, F., and Rinehart, J. F. Am. J. M. Sc. **186**: 694, 1933.

Data are presented of studies made on the relationship of diet and nutrition to anemia in 10 cases of chronic idiopathic hypochromic anemia associated with hypochlorhydria or achlorhydria and defective diet.

The response of the bone marrow, as determined by the hemoglobin and production of erythrocytes, to iron administered in large daily oral doses in the form of iron and ammonium citrate (U.S.P.) was compared to the response in patients on an "iron rich" diet. In addition, a comparison was made of the response of the bone marrow to a diet rich in iron and to a meal previously digested in vitro with hydrochloric acid and commercial pepsin.

The bone marrow responded rapidly and excellently to large doses of inorganic iron, but there was no evidence of increased hematopoiesis after the ingestion of an "iron rich" diet for a long period of time. There was, however, a rapid and satisfactory increase in concentration of hemoglobin, production of red cells, and a slight reticulocyte response following the administration of predigested meals.

It is concluded, from these studies, that chronic idiopathic hypochromic anemia is one presumably due to a deficiency of iron wherein gastric dysfunction leads to failure in utilization of organic (dietary) iron.

EOSINOPHILIA, Induction of in Normal Animals, Banerji, N. Am. J. M. Sc. **186**: 689, 1933.

An elevation in the relative number of eosinophiles was induced in rabbits subjected to increased carbon dioxide tension of the blood, either by intravenous administration of carbon dioxide solutions or by exposure of the animal to an atmosphere containing increasing amounts of this gas.

From this result, it is concluded that an alteration in the acid base equilibrium of the body appears to incite an eosinophilia.

Further studies, it is hoped, will lead to a better understanding of the mechanism of this alteration.

CANCER, The Reaction of the Arterial Blood in, Dickinson, S., and Havard, R. E. Brit. J. Exper. Path. **14**: 394, 1933.

The results of previous workers are summarized and discussed.

The technic is described of collection of samples of arterial blood from patients with and without malignant diseases, and the measurement of the P_H of the blood at 37° C., by two independent glass electrodes.

The results support the conclusion that the blood of patients with malignant disease of the mouth or skin, or with secondary glands, is not more alkaline than that of patients free from malignant disease.

An experiment is described that demonstrated that momentary alterations of respiration can affect the P_H of the blood.

ENTERITIS, Polytropous (Acute Infectious Gastro-Enteritis, Spencer's Disease), Wildman, H. A. Arch. Int. Med. **52**: 959, 1933.

There apparently exists quite generally a clinical entity which has erroneously been called gastrointestinal influenza, food poisoning, ptomaine poisoning, biliousness, acute autointoxication, dietary indiscretion and the like. Considerable information concerning the etiology, epidemiology, pathogenesis, symptomatology and differential diagnosis of this condition has been gathered from observations on more than a thousand cases seen within the past seven and a half years. A distinctive name is now proposed. The present report is based on a study of about 750 college students who had from one to six or more attacks between September, 1927, and June, 1932. Much more work is needed to settle some points, particularly those concerning the etiologic agent and the pathologic condition which it produces.

ACETANILID, Influence of Caffeine on Effects of, Higgins and McGuigan. J. Pharmacol. & Exper. Therap. **48**: 276, 1933.

Study upon animals of the effects of acetanilid plus caffeine did not present evidence of increased acetanilid toxicity. Mice receiving daily in their drinking water 50 per cent of the fatal hypodermic dose of acetanilid and 20 per cent of the fatal hypodermic dose of caffeine showed practically no effects from the drugs. The enormous dose with their food (0.5 per cent acetanilid and 0.5 per cent acetanilid plus 0.1 per cent caffeine or 650 mg. per

kilogram body weight) served only to delay growth in mice. Caffeine seemed only to lessen the toxicity. After six weeks the animals were placed on normal diet and rapid gain in weight followed.

Three hundred and twenty-five mg. acetanilid plus 62 mg. caffeine per kilogram per day had little or no effect on growth.

In rabbits the fatal dose of acetanilid is about 1.5 gm. per kilogram. In anesthetized dogs fatal doses cause respiration to stop before the heart. In therapeutic amounts caffeine would not add to the toxicity of acetanilid.

SYPHILIS, Conjugal, A Statistical Study, Decker, H. B. Am. J. M. Sc. **187**: 111, 1934.

There is presented an investigation of the records of 376 families in which syphilis was present in at least one member of the conjugal union.

Sixty husbands and 98 wives were syphilitic with nonsyphilitic spouses.

In 218 families both husband and wife were syphilitic.

It is concluded that the type of syphilis depends upon the individual rather than upon any special strain of treponema.

Late syphilis is relatively noninfectious. Infectivity varies inversely with the duration of the disease.

GRANULOMA INGUINALE, Further Studies on the Etiology of, De Monbreun, W. A., and Goodpasture, E. W. Am. J. Trop. Med. **13**: 447, 1933.

A gram-negative bacillus belonging to the aerogenes group has been isolated from the lesions of two cases of granuloma inguinale, and from the feces of two out of four other cases of the disease.

This bacillus reproduces exactly the morphological characteristics of the Donovan microorganism, including the typical Donovan body which appears as a secondary phenomenon in older cultures in blood media.

Cultures of this bacillus have failed to reproduce the specific lesions of granuloma inguinale when inoculated into lower animals and into man.

The Donovan microorganism apparently increases in mononuclear cells in tissue cultures in the primary explants from human lesions, but has failed to grow in subcultures.

Notwithstanding failure to reproduce granuloma inguinale experimentally with cultures of the bacillus described, the hypothesis that this disease is primarily a chronic intestinal infection with a member of the aerogenes group of bacilli, with secondary infection of the skin from constant fecal contamination is advanced as worthy of further investigation.

ACTINOMYCETES, Modified Sabouraud Medium for Cultivation of Acid-Fast, Scudder, S. A. Science **79**: 16, 1934.

The following medium was found satisfactory:

Maltose, 4 per cent; Difco peptone, 1 per cent; flaked agar, 1.8 per cent; dissolved in unfiltered beef heart or veal infusion instead of water. No adjustment of reaction is necessary. If desired, glycerin and other carbohydrates may be added. The use of the medium as slants favors the development of acid-fastness in about four days. Simultaneously with the appearance of the acid-fast portions of the growth, a grayish-brown powdery substance develops upon the upper portion of the slant.

MICROSCOPY, Using a "Dry" Microscope Objective on Uncovered Objects, Sauer, F. C. Science **78**: 537, 1933.

By attaching a cover glass temporarily to the lens (by a film of cedar oil, mineral oil, or water) the study of uncovered objects is greatly improved.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Der Krebs, seine Entstehung und Erklärung*

A THEORETICAL discussion which defines cancer as a biologic result of the disturbance of the three factors which regulate cell life, that is (1) a disturbance of the life forces resident in the cell, (2) a lack of normal hormonal influence or excess of pathological dishormonal influence, and (3) lack of the trophic innervation of the vegetative nervous system. Disturbance of all three factors causes the revolutionary cancerous transformation of the cell; disturbance of two factors produces the precancerous stage.

Die normale und pathologische Physiologie der Milz†

THE author modestly acknowledges the scanty and conflicting evidence concerning the functions of the spleen and states that he has spent years in personal attempts to decide certain disputed points. He hopes that his book may be of assistance to others working in this dark and difficult field. One of the anomalies is the fact that removal of the spleen in man causes no detectable symptoms while its removal in rats and many other experimental animals results in a few days in a Bartonella infection and a rapidly fatal anemia. Apparently most laboratory animals harbor a latent Bartonella infection which is normally held in check by the spleen but which runs wild after splenectomy. In the various chapters the spleen is considered as a blood reservoir, as a hematopoietic and hemolytic organ, as a protection against infections and tumors, as it influences the digestive tract and metabolism, and as an organ of internal secretion.

Das Chlorophyll als Pharmakon‡

CHLOROPHYLL, the common green respiratory pigment of the plant world, has recently been studied intensively by Willstätter and others, and it has been found to be closely related in chemical structure to hemoglobin, the common red respiratory pigment of the animal world. The nucleus of each consists of a ring structure made up of four pyrrole groups and a metal, iron in hemoglobin and magnesium in chlorophyll. In cases of anemia it would seem a natural and logical indication to supply this pyrrole nucleus for the building of new blood. In fact, both blood and chlorophyll have been so used but the results are no better than are obtained by the administration of simple forms of iron. Bürgi, however, finds that chlorophyll has a very general tonic effect on all the organs and functions of the body; it tones the heart and blood vessels, it increases peristalsis, it lessens the fatigue of nerve muscle preparations, etc. He finds that rabbits fed green vegetables are brighter and more active, and we all know that the old cow is rejuvenated when she gets green grass in the spring. Green vegetables, however, contain also carotinoids, vitamins and other products, but Bürgi finds that extracted chlorophyll has this same tonic effect and is more completely absorbed than is chlorophyll taken in the form of green vegetables.

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*Der Krebs, seine Entstehung und Erklärung; eine biologische Studie. (Cancer, Its Origin and Explanation; a Biological Study.) By A. W. Kukowka. Wilhelm Maudrich, Vienna. 1932.

†Die normale und pathologische Physiologie der Milz. (The Normal and Pathological Physiology of the Spleen.) By Ernst Lauda. Urban & Schwarzenberg, Berlin. 1933.

‡Das Chlorophyll als Pharmakon. By Emil Bürgi. Georg Thieme, Leipzig, 1932.

The Anatomy of the Rhesus Monkey*

CONSIDERING the extensive use of the rhesus monkey as a laboratory animal, it is strange that some such publication as this volume has not hitherto been available.

While not, strictly speaking, a textbook, the volume nevertheless represents the first comprehensive description of the anatomy of the old world monkey and as such may justly be regarded as a pioneer in this field.

It consists of eighteen chapters representing the studies of competent contributors thoroughly familiar with their subject.

Each contributes a detailed study of a definite field so that the volume as a whole covers the subject in its entirety.

It is, of course, not to be expected that the volume should approximate in character the textbooks of human anatomy because of the limited numbers of dissections and anatomical investigations upon which its studies are based.

Nevertheless, the research worker and the student of comparative anatomy will find in its pages much that has not before been subjected to extensive study or comprehensive description and while future studies may, to some extent, lead to modification or revision of this or that section or portion of a section, the work represents a contribution of great value and definite utility.

Very wisely, it seems to the reviewer, and contributing to the usefulness of the book, the B. N. A. nomenclature, rather than that of comparative anatomy, has been used throughout.

An appendix contains an excellently written and illustrated account of the housing and care of monkeys which should prove most useful.

The contributors, editors, and publishers may be congratulated upon the production of a useful volume.

A System of Clinical Medicine†

THE fact that this book, addressed particularly to students and practitioners, has reached a ninth edition is prima facie evidence of its practical character and usefulness which becomes evident even with the most cursory perusal of its pages.

There are many books concerned with clinical medicine and the problems confronting the practitioner, all having their place and value. Few, however, will in a broad sense possess any greater utility than Savill's.

This is largely due to the somewhat different, and original plan upon which it is based in presenting the subject from the purely clinical standpoint, the bedside point of view, as it were, in which the problem confronting the physician is to correlate the symptoms observed with their underlying cause.

The discussion of each condition, therefore, is approached from the following angles:

First, a discussion of the symptoms and their possible causes in which is presented a comprehensive outline of the signs indicative of disease in the organ or region under discussion, as well as a discussion of the fallacies incident to their detection and a differential account of their various possible causes.

Second, a discussion of the physical signs in that region and the methods of eliciting them.

Third, a clinical classification of the various diseases affecting the region in question and a discussion of the various diseases arranged in accordance with their clinical relationships.

By this arrangement the reader is presented with a very useful clinical index of diseases and finds himself reading the diagnosis, prognosis, and treatment of the disease at

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*The Anatomy of the Rhesus Monkey. Edited by Carl G. Hartman and William L. Straus Jr. Cloth, 122 figures. The Williams & Wilkins Co., Baltimore, Md.

†A System of Clinical Medicine. By Thomas Dixon Savill, M.D. Ninth edition, cloth, pp. 1063, 163 illustrations, 6 colored plates. William Wood and Co., Baltimore, Md.

hand and also, in immediate proximity finds a description of conditions which clinically and pathologically often more or less resemble it and thus may be mistaken for it.

For any degree of success the carrying out of such a plan necessarily requires a foundation of broad and digested experience. That Dr. Savill has been successful in his project the volume itself bears witness.

The present edition, thoroughly revised and brought up to date, brings to the practitioner and student an exceedingly useful and well-written presentation.

Food-Borne Infections and Intoxications*

WHILE food poisonings and food infections are relatively unimportant as a cause of death, a sufficient number of outbreaks occur to warrant an understanding of their nature and mechanism.

In this book, a companion volume to the author's previous text upon *The Microbiology of Foods*, food infections and food intoxications are presented in a thoroughly comprehensive and satisfactory manner.

Those interested in this subject will find in this book practically the information available concerning every type of food infection or poisoning on record. Under each heading will be found illustrative case reports and records of outbreaks, the bibliography being exceptionally full and complete. The style is clear, simple, and eminently understandable.

This book and its companion should be of interest, not only to the physician who may be confronted with outbreaks of this character as well as with others simulating food poisoning but really something else, but especially to health officers and laboratory workers who may be called upon to study such outbreaks.

It can be recommended as a thorough and comprehensive study of a subject not always clearly understood.

Textbook of Pathology†

THIS well-known book requires no introduction, having served as a standard text for the last decade.

In this, the third edition, will be found a number of changes and additions, not only in the text, some portions of which have been entirely rewritten, but also as concerns the illustrations, a number of which are new and all of which in general will be found adequate.

While following in general the common division of the subject into General and Special Pathology, this distinction has not been overemphasized. Based upon the author's experience as a pathologist and teacher, the volume will be found practical and of value, not only to the students to whom it is particularly addressed, but also to the clinician and, as heretofore, as a work of reference.

There is an adequate index.

Die Spezifizität der serologischen Reaktionen‡

THIS is an attempt to bring together, coordinate, and interpret for German readers the results of his own investigations and those of his coworkers and to discuss the very difficult problems of biological specificity, especially from the chemical standpoint. He takes up in order the specificity of proteins, of cell antigens and antibodies; then the specificity of serological reactions with artificial complex antigens and finally the specific cellular substances such as the specific carbohydrates and lipids. It has copious references and an index.

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*Food-Borne Infections and Intoxications. By Fred Wilbur Tanner, Ph.D., Professor of Bacteriology, University of Illinois. Cloth, pp. 439. The Twin City Printing Co., Champaign, Ill.

†Textbook of Pathology. By Robert Muir, Professor of Pathology, University of Glasgow. Third edition, cloth, pp. 957, 546 illustrations. William Wood and Co., Baltimore, Md.

‡Die Spezifizität der serologischen Reaktionen. By K. Landsteiner. Julius Springer, Berlin, 1933.

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EDITORIAL

The Hazards of Statistical Interpretation

A SURVEY of the effect of the economic depression upon sickness has been undertaken by Perrott, Collins and Sydenstricker.[1] The completed analysis will be based upon the records of illness in 1933 as compared with the economic history from 1929 to 1932, in more than 12,000 families in eight large cities, a group of coal mining communities, and a group of cotton mill villages. The authors' preliminary report presents the preliminary results in three of the large cities, Birmingham, Detroit, and Pittsburgh. This comprises a group of 2,566 families, 11,330 individuals. They find a definitely higher incidence of disabling illness among individuals in the lower income classes than among those with higher incomes. The highest sickness rate is reported in the group which in 1929 had been in reasonably comfortable circumstances but which in 1932 had dropped to relative poverty. The sickness rate in this group is 60 per cent higher than among those who in 1929 were in comfortable economic

state and who maintained that state through 1932. The rate was even higher than that for the "chronically poor" group, those who were poverty stricken in 1929 and 1932.

The rate of disabling illness in families of the unemployed was 25 per cent higher than that in families containing part-time workers and 39 per cent higher than that among families of full-time wage earners.

These conclusions are such as one would anticipate, and could easily form the basis for generalizations regarding the effect of a financial depression on sickness in general. The authors, however, very wisely emphasize that conclusions should be drawn only on the material studied. They make no attempt to draw broad implications and caution the reader to exercise similar restraint. The group is relatively small and does not necessarily represent a cross-section for the United States. They also point out that specific interpretation of increased illness rate in terms of impaired vitality cannot be made without knowledge of the nature of the illnesses experienced.

In this study the results were what one would expect. In another recent analysis[2] they were the reverse. Deaths from tuberculosis in 28 states surveyed were lower in 1932 than in any of the preceding four years. The fall in rate has followed the regular established curve for tuberculosis, apparently uninfluenced by the depression. The rate per 100,000 in 1928 was 77.3; in 1932, 60.4.

A very interesting example of the difficulty of drawing statistical conclusions from relatively small figures is reported by C. M. Smith in his study of housing conditions and respiratory disease in Glasgow.[3] This also was in a measure an economic survey. The prevalence of respiratory disease was studied in two Glasgow areas, one a poor-class quarter, a slum district, and the other a rehousing area. The latter corresponds to our model tenement developments. The survey covered an entire year. The inhabitants of the two areas, numbering about 1,000 each, were in comparable economic states, but the housing facilities in one group were presumably much more sanitary than in the other.

To our surprise we find that there was a greater morbidity from acute respiratory disorders in the rehousing area than in the slum quarter. Here we are presented with an apparent paradox to the anticipated, and the explanations presented are based upon isolated observations rather than figures. Smith states that the results cannot be taken as evidence that improved housing conditions have been without effect on the prevalence of respiratory disease for the following reasons. In the slum district 20 per cent of the population were foreigners, chiefly Jews and Lithuanians, among whom there was less unemployment and dietary habits were better than in the rest of the population. The willingness of informants to report cases is a factor, and the author believes that reporting of illness was more accurate in the model tenement group. Theoretically, provision in the model tenement district allowed for fewer persons per room, there being only about one-fifth as much overcrowding. As a matter of fact a large proportion of the rehoused population, not long removed from the slums, continued to use just one of their several rooms for sleeping, especially in cold weather. Therefore, during the night, crowding was just as prevalent

in the rehousing district. The study illustrates beautifully some of the difficulties encountered in undertaking field surveys and drawing conclusions therefrom.

There is perhaps a natural tendency in making any statistical analysis, however small, to accept as accurate those figures which appear to substantiate what one's judgment or one's theory leads one to believe to be correct. Too often one is inclined to overlook the tremendous number of units required before all variables become negligible. The requisite total number varies, naturally, with the subject matter studied. Also, unfortunately, in many types of surveys in which statistics must be employed, sufficiently large numbers are actually not available.

This should not rule out the possibility of recourse to statistical methods, but when these are used one would do well in drawing conclusions, even though they appear entirely correct, to adopt the cautiousness manifested by Perrott, Collins and Sydenstricker, in applying conclusions only to the material studied and avoiding unjustified generalizations.

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2. Public Health Reports **48**: 479, 1933.
3. Smith, C. M.: Housing Conditions and Respiratory Disease. Morbidity in a Poor-Class Quarter and in a Rehousing Area in Glasgow, Medical Research Council Special Report Series, No. 192 (London), 1934.

—*W. T. V.*